QUANTITATIVE VARIATION IN PHYTOPHTHORA CACTORUM (LEB.AND COHN.) SCHROET.

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CONTENTS

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To Annie

Chapter 1. General Introduction

The Oomycetes have been studied for some 130 years, but we are still ignorant of many aspects of their physiology, genetics and general biology. This is particularly true of the genus Phytophthora, many species of which are important plant pathogens, e.g. P.infestans, the causal organism of late blight of potato. The genus includes both homothallic and heterothallic species. The heterothallic species have two mating types which must pair for sexual reproduction to occur. Pairing strains of a single mating type does not result in oospore formation. Within each mating type there is variation in sexual expression. Some strains produce oogonia, others antheridia, while others behave bisexually, the type of gametangia formed being dependent upon the opposing strain (Galindo and Gallegly,1960). This behaviour is similar to the phenomenon of relative sexuality observed in Achlya bisexualis and A.ambisexualis (Barksdale,1960). The distinction between homothallic and heterothallic species is not clear cut as some isolates of heterothallic species may form oospores in unpaired cultures (Galindo and Gallegly,1960; Brasier,1972).

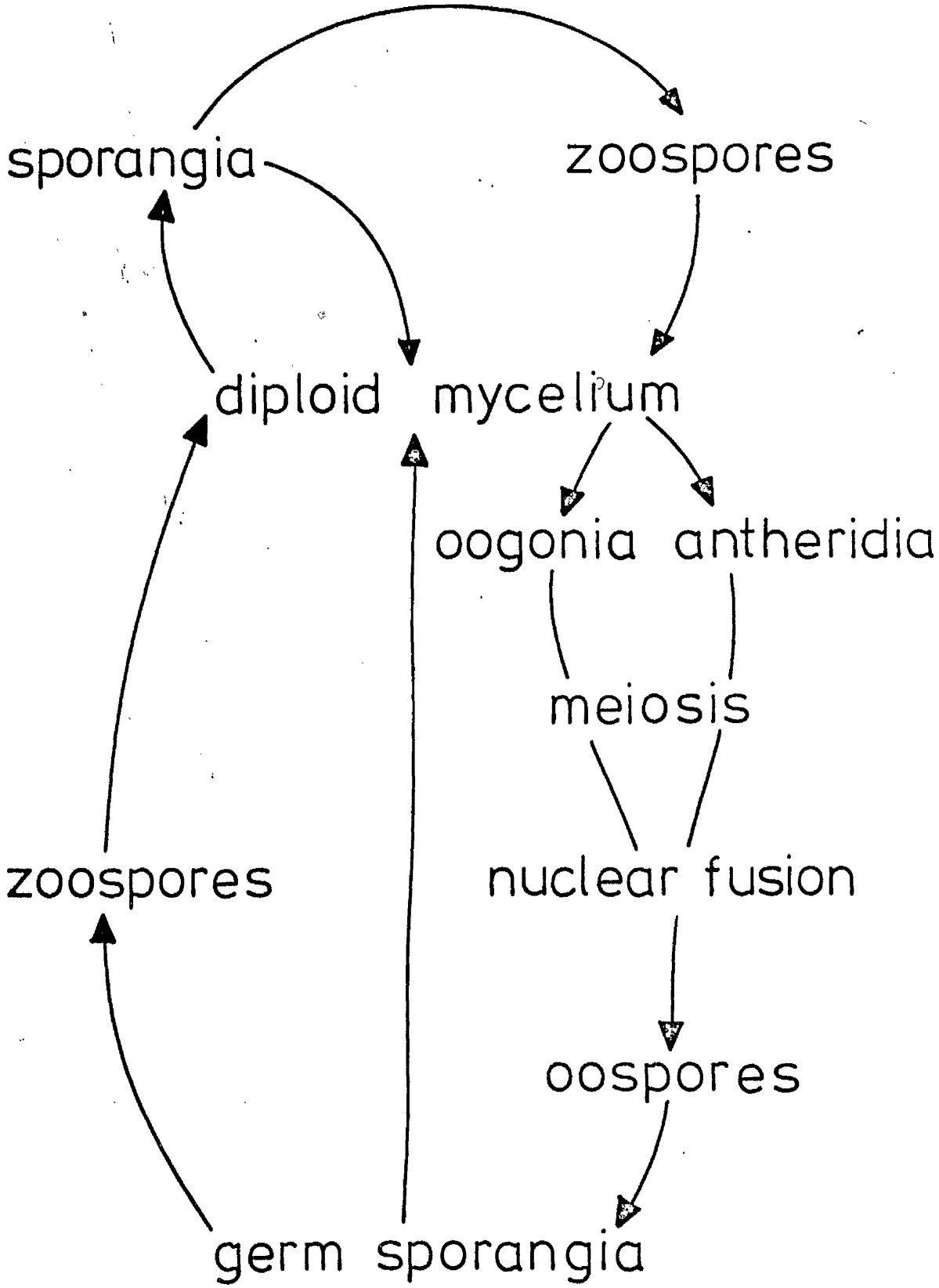


Fig. 1. Life cycle of P. cactorum.

P.cactorum is homothallic and therefore only one type of mating is possible. Antheridia and oogonia are produced side by side on homokaryotic mycelium and meiosis in the gametangia is followed by plasmogamy, karyogamy and the formation of a single oospore (Blackwell,1934(a);Sansome,1966;Galindo and Zentmyer, 1967;Huguenin and Boccas,1970). Alternatively the fungus may reproduce asexually by means of sporangia and uninucleate zoospores (Blackwell,1943(a)),(Fig.1).

P.cactorum has a vast host range and can cause rots of fruit,leaf,stem and root (Waterhouse and Waterston,1966). Its destruction of timber and fruit crops in temperate regions is of considerable economic importance. Rapid dispersal and short term survival of the pathogen is acheived by the asexual spores,whilst the oospore,which may remain dormant for many years, is the agent of long term survival. Also,the oospore is the site of genetic recombination (Galindo and Zentmyer,1967;Romero and Erwin,1967,1969;Satour and Butler,1968;Polach and Webster,1972) and therefore provides a means by which new strains may evolve. But as P.cactorum is homothallic, new genetic variability must be continually introduced by process of mutation, or, presumably, heterokaryosis.

Although the genus is of such great economic importance, progress in the study of its genetics has been extremely slow in comparison with the higher fungi: Yeast, Neurospora, Aspergillus, Schizophyllum and Coprinus. This has undoubtably been due to the

difficulties encountered in obtaining nuclear mutants. Also, the oospores of many isolates will not germinate well and as a consequence the interpretation of induced and natural variation has often been unreliable.

There need be no excuse for studying variation in Phytophthora for we understand little of the genetic mechanisms that determine pathogenic and cultural variation in the genus (Gallegly,1970). We know from recent cytological evidence (Sansome,1965,1966;Huguenin and Boccas,1970;Sansome and Brasier,1973) that the genus is probably diploid. This view is supported by genetical evidence on the nuclear cycle in P.drechsleri (Shaw and Khaki,1971) and P.cactorum (Elliott and MacIntyre,1973). However, the numerous reports of pathogenic variation (e.g.Black,1960;Paxman,1963;Caten, 1970;Polach and Webster,1972) and cultural variation (e.g.Buddenhagen,1958;Clarke and Robertson,1966;Caten and Jinks,1968) in the genus are largely unexplained as the oospores of most species will not germinate well.

It is perhaps necessary to justify the choice of P.cactorum for this work in preference to other species. With respect to genetical studies it is probably the easiest species to handle under laboratory conditions.

It can complete its life-cycle in culture in a period of about five weeks and produces zoospores and oospores, both of which are uninucleate and germinable (Blackwell, 1943(a),1943(b);Shaw,1967). Sexual progenies are entirely the result of selfing - there is no uncertainty in interpreting results as is the case with heterothallic species where it appears that some selfing occurs but its extent is unknown (Sansome,1970;Brasier, 1972). However, to work with a homothallic species is a disadvantage in that hybrid progenies cannot be obtained easily. To perform crosses with P.cactorum would necessitate the incorporation of nuclear auxotrophic mutations into the desired strains, forcing a heterokaryon and selecting hybrid oospores. As only a few auxotrophic strains are available between which heterokaryons have not been formed, obtaining crossed progenies of P.cactorum is not feasible at present.

P. infestans has often been chosen for detailed studies of natural variation in Phytophthora, but as sexual progenies of this species cannot be easily obtained, the work has been limited to observations on asexual variation (Jeffrey,Jinks and Grindle,1962; Jinks and Grindle,1963;Paxman,1963;Caten and Jinks, 1968;Upshall,1969(a)(b);Caten,1970,1971). Furthermore, meaningful analysis of asexual variants is impossible without being able to observe their pattern of inheritance through the sexual cycle. Clearly,

P.cactorum is much more suitable for this kind of study.

Within the limitations of the material, the aim of this project is to determine the range and mechanism of variation in cultural characters of P.cactorum as revealed by methods of asexual and sexual propogation. It is not yet possible to distinguish cytoplasmic and nuclear sources of variation by a heterokaryon test although information on this point may be apparent from comparisons of zoospore and oospore progenies. If, however, the cytoplasmic differences do not contribute to the variation between oospores then it should be possible to separate the components of the genetic variance by comparing a large number of S1 and S2 progenies (Mather and Jinks,1971,p.271).

The work described here comprises selection experiments designed to provide information on the variation within progenies and the heritability of the character as shown by the response to selection. Selection from both zoospore and oospore progenies has been carried out to form a basis for the comparison of asexual and sexual sources of variation.

The use of selection experiments to study Quantitative variation in fungi has been successfully applied to Neurospora crassa (Pateman,1959;Pateman and Lee,1960;Papa,Srb and Federer,1966) and

Schizophyllum commune (Simchen,1966;Connolly and Simchen,1968). However, P.cactorum differs from these and most other fungi in a number of characters which distinguish the Oomycetes, i.e. the possesion of characteristic zoospores, the oospore, the composition of the hyphal wall and the nature of the life-cycle. Therefore one might expect this work to reveal mechanisms of variation not yet demonstrated for the fungi.

Chapter 2. Materials and Methods

Part I Strains

The majority of the experiments on variation in P.cactorum have been performed on strain P94, supplied by Dr.G.W.F.Sewell. This strain was isolated from apple rootstock (MM106) at Wisbech, Cambridgeshire in October 1970. In some experiments strain P205 of P.cactorum (supplied by Dr.G.W.F. Sewell) was used.

Part II Media

MINIMAL MEDIUM (MM)

MM was prepared from a concentrated solution containing sucrose,40g; L-asparagine,4g; KH_2PO_4,2g; $MgSO_4.7H_2O$,1g; calcium glycerophosphate $\frac{1}{2}H_2O$,1g; NaCl, 0.47g; trace element solution, 4ml; thiamine

hydrochloride,4mg; and distilled water,1L (Elliott and MacIntyre,1973). The trace element solution contained $Na_2B_4O_7 \cdot 10H_2O$, 88mg; $CuSO_4 \cdot 5H_2O$, 393mg; $Fe_2(SO_4)_3 \cdot 9H_2O$, 910mg; $MnCl_2 \cdot 4H_2O$, 72mg; $Na_2MoO_4 \cdot 2H_2O$, 50mg; $ZnSO_4 \cdot 7H_2O$, 4403mg; ethylenediaminetetra-acetic acid disodium salt, 5g; and distilled water,1L(Elliott, 1972). For use the concentrated MM solution was diluted 1in 4 and solidified with oxoid agar No.3. 10 g/l.

MM was used in all growth rate determinations and morphological comparisons.

PLAIN AGAR (PA)

PA consisted of 1% Oxoid agar No.3 in distilled water and was used in the production of zoospores and the germination of oospores.

STANDARD MEDIUM AGAR (SMA)

SMA, which supports good colonial growth, has been used in the preparation of innocula. It contained 1 part concentrated MM, 2 parts of an aqueous extract of peas and 1 part distilled water, solidified with 1% Oxoid agar No.3. The aqueous extract of peas was prepared by bringing 300g of frozen garden peas in 1L distilled water to the boil and filtering through muslin (Shaw and Elliott,1968).

PEA MEAL AGAR (PMA)

This medium which supports luxuriant growth, was used in the production of zoospores. It consisted of 300g of frozen garden peas blended with 1L of distilled water and solidified with 1% Oxoid agar No.3 (Shaw and Elliott,1968)

STANDARD MEDIUM AGAR + OAT EXTRACT

To obtain a good crop of oospores the fungus was grown on SMA to which 1% of a light petroleum extract of oats was added before autoclaving (Shaw,1965).

Part III Culture methods

Innoculum, 4mm in diameter was obtained using a No.1 cork borer and the fungus was normally grown on solid media in 9cm glass petri dishes and incubated in the dark at 25°C. Stock cultures were maintained in one ounce bottles containing MM slopes. To prevent dessication of the medium, 10ml of sterile parafin oil was added to each one ounce bottle and the rubber seal removed.

Part IV Obtaining Zoospore Progenies

To obtain a good crop of sporangia, plates of PMA were innoculated centrally and incubated for four days. Mycelial strips, free of medium, were cut from

the resultant colonies and three strips transferred to each of a number of plates of PA. After three days incubation the strip cultures contain numerous sporangia that are capable of zoospore release.

Zoospore liberation was acheived by transfering the strip cultures to 15°C and flooding each petri dish with 5ml of sterile distilled water at 15°C. After 30 minutes and a further 15 minutes, 2.5ml of a 6% sucrose solution was added to the cultures, bringing the concentration of sucrose in the suspending medium to 3%. At this point the zoospore suspension was harvested (Shaw,1965).

When isolating individual zoospores the suspension was plated on SMA and after 12 hours incubation, individual sporelings were reisolated onto fresh plates of SMA, distributed four per plate.

Part V Obtaining Oospore Progenies

To produce oospores, plates of SMA + oat extract were innoculated centrally and incubated for at least 21 days to allow the spores to form and mature. Three such cultures were blended with about 100ml sterile distilled water in an Atomix (Measuring and Scientific Equipment Ltd.) for 10 mins at half speed. 10ml of the suspension was transferred to a 12ml glass centrifuge tube and spun at about 2000g for 10 mins.

Water, agar and hyphal fragments, forming the uppermost layers were removed to leave a spore pellet which was washed five times by centrifugation and resuspension in sterile distilled water. The washed spores were spread onto PA plates and individual oospores picked up on the tip of a fime platinum needle and transfered to fresh plates of PA. 4 oospores were placed on each PA plate and the plates exposed to continuous light from a bank of 'Gro-Lux' fluorescent tubes at a distance of 20cm. After a period of 10 days, germination was scored and germinated oospores transferred to petri dishes of SMA (four sporelings per plate) to allow establishment to occur (Elliott and MacIntyre, 1973).

Oospores most commonly germinated to produce a short sporangiophore bearing a single sporangium and successful establishment usually resulted from proliferation of the sporangiophore. However, death of germinating oospores was quite common and occurred at one of three stages of development : (1) prior to germ tube emergence, (2) at the germ sporangium stage and (3) subsequent to developing a germ mycelium.

Part VI Growth rate determination

Selection for fast and slow growth rate in Neurospora crassa (Papa, Srb and Federer, 1966) and

Schizophyllum commune (Simchen and Jinks,1964) has demonstrated the suitability of growth rate as a character with which to study quantitative variation. It was chosen for the selection experiments on P.cactorum as measurements can be easily obtained and in comparison with such characters as growth rate in liquid culture and fertility, there is little variability between replicate determinations.

To reduce the variation between experiments as far as possible, standard practice was adopted in the preparation of cultures and the taking of measurements. Growth rate was always determined on MM, which was chosen in preference to an undefined medium, the contents of which could vary undetected. Also, a complete medium such as SMA would contain the chemical requirements for oospore formation (Elliott,Hendrie, Knights and Parker,1964). Therefore, if oospores are allowed to form during the growth rate determinations, it is possible that sexual reproduction will occur between generations of selection.

In studies of quantitative variation, the growth rate of P.infestans on a medium containing a rye grain extract (Caten and Jinks,1968), of Neurospora crassa on a minimal medium (Papa,Srb and Federer,1966) and of Schizophyllum commune on 2% malt extract (Simchen and Jinks,1964) were determined using growth tubes (Ryan, Beadle and Tatum,1943). But as P.cactorum is slower

growing than these species; its growth rate was measured in 9cm glass petri dishes (BS 611). In comparison with growth tubes, petri dishes have several advantages. They are easily obtained, occupy little space, and permit observations of colony morphology, particularly with respect to sectoring. Also, growth in a single petri dish can be determined as the mean of several measurements of colony size, whereas a single growth tube can yield only one measurement.

Each petri dish contained 16ml of MM poured with a Zippette dispensor (Jencons,Scientific Limited) and all the plates comprising one experiment were prepared from a single batch of medium. The innoculum was taken from five day old colonies on SMA, inverted and placed in the centre of each petri dish. After a suitable period of incubation, measurements to the nearest mm of greatest and least colony diameter through the innoculum were taken. From these measurements, linear growth rates were calculated by dividing the mean radial increase of each colony by the number of days incubation.

When using petri dishes, growth rate determinations vary somewhat dependant upon the stage of development of the colonies from which measurements are taken. Therefore a number of experiments involving either zoospore, oospore or mass hyphal

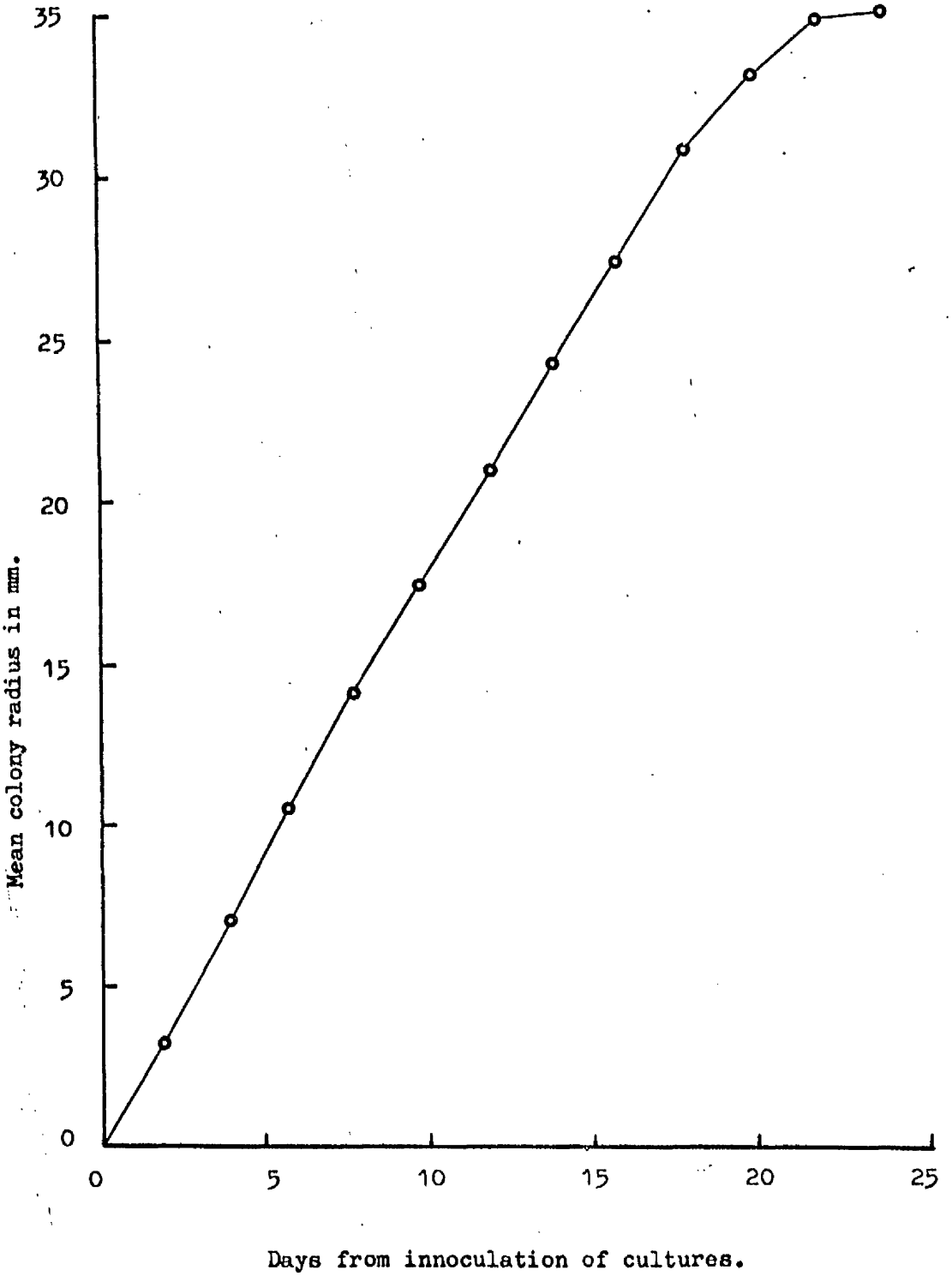


FIG.2, RADIAL INCREASE OF MASS HYPHAL ISOLATES OF STRAIN P94.

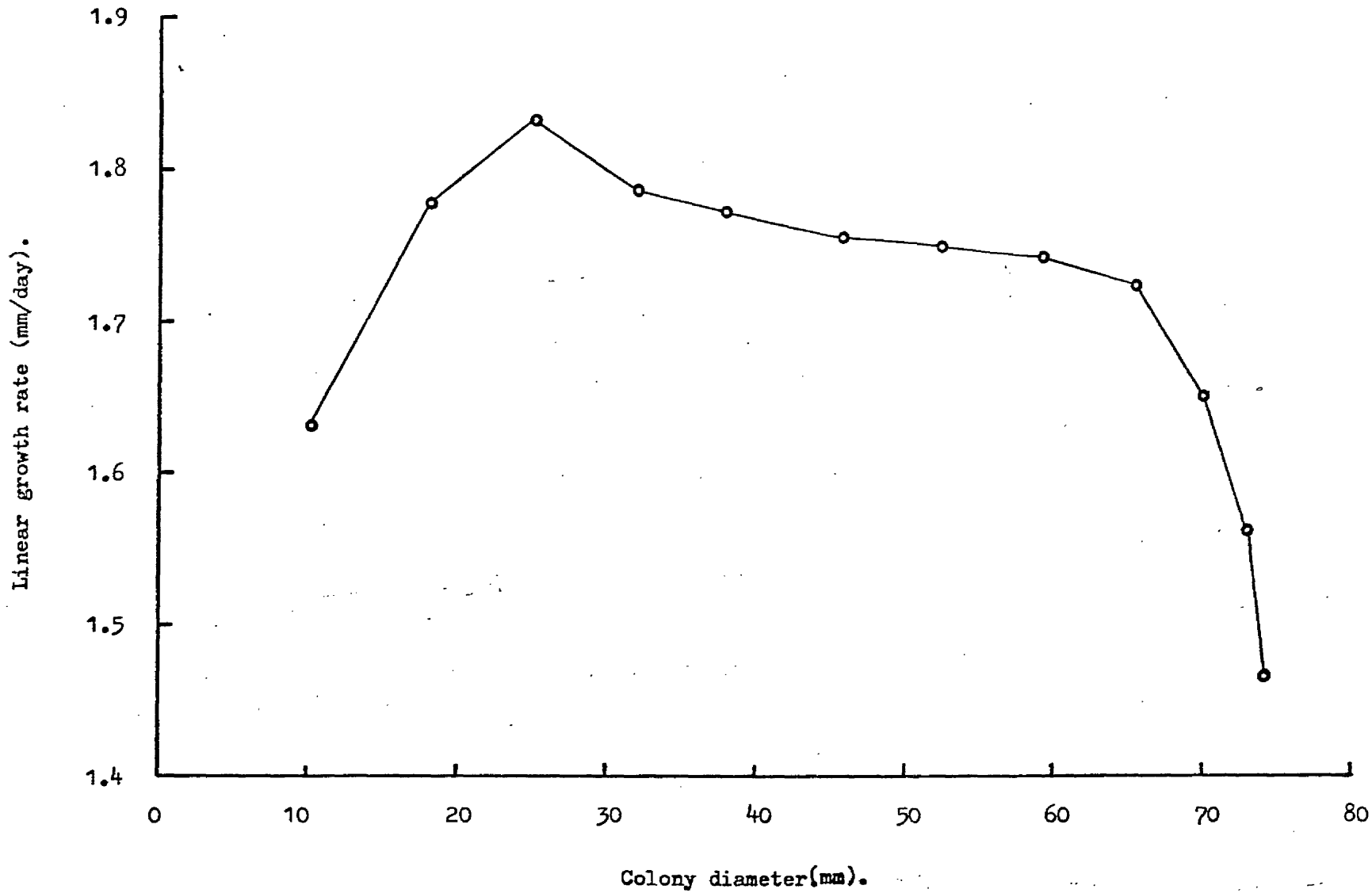


FIG.3, SUCCESSIVE DETERMINATIONS OF GROWTH RATE ON COLONIES OF INCREASING DIAMETER.

derivatives were performed to determine the nature of growth in petri dishes and the most suitable stage at which to take measurements of colony size. The results obtained using different methods of propogation were similar and can be illustrated by one experiment in which successive measurements were made every two days on 13 mass hyphal isolates of strain P94 (Fig2). Radial increase of the colonies was roughly linear with time up to a point about 10mm from the edge of the petri dish. Beyond this point, further extension was restricted by the size of the petri dishes. In Fig.3 the growth rates have been calculated from measurements made every two days and plotted against colony diameter. Each point on the graph represents the mean growth rate of the colonies up to the time they were measured. The important feature of the curve is that growth rate determinations do not vary greatly if they are obtained from colonies of diameters 35-60mm. It follows that if the best comparison between experiments is to be acheived, then measurements must be taken from colonies within this size range. As a result all measurements were taken from colonies of approximately 50mm diameter, or where an experiment consisted of isolates of varying growth rate, they were all measured when the faster isolates had reached this size.

AGE OF CULTURES IN DAYS	MEAN COLONY DIAMETER IN MM	MEAN GROWTH RATE	S.E. OF GROWTH RATE MEASUREMENTS
1.94	10.3	1.63	0.038
3.98	18.2	1.78	0.021
5.77	25.1	1.83	0.019
7.87	32.1	1.79	0.014
9.81	38.8	1.77	0.015
11.92	45.8	1.75	0.008
13.91	52.6	1.75	0.011
15.87	59.3	1.74	0.011
17.92	65.7	1.72	0.010
19.98	70.2	1.65	0.009
22.05	73.0	1.56	0.011
23.92	74.3	1.47	0.006

Table 1. Data derived from successive measurements on 13 mass hyphal isolates of strain P94.

More information can be derived from the experiment on mass hyphal isolates of P94 from the standard errors of each set of determinations (Table 1). These values, although large for colonies of small size, do not vary for larger colonies when colony size increases. Therefore within the size range from which measurements will be taken, errors in growth rate determination do not appear to be correlated with colony size. The consideration must be extended to include isolates of different growth rates. This may be done by comparing the data of Table 6, which contains error values obtained from progenies differing in mean growth rate. For these progenies, no correlation between mean growth rate and the differences between replicate measurements can be detected.

One can conclude that error in growth rate determination does not appear to be correlated with colony size for colonies larger than 25mm in diameter, or with colony growth rate, and therefore the use of untransformed growth rate measurements will not result in serious distortion of the analysis of variance.

Part VII Oospore Counts

To determine whether or not selection for growth rate was accompanied by changes in fertility, oospore

counts were performed on representitive isolates obtained from the selections. The method of counting was very similar to that used by Elliott (1968). The isolates were cultured in 5cm 'Monax' petri dishes, each of which contained 4ml MM poured with a Zippette dispensor (Jencons, Scientific Limited). After the MM had solidified, cholesterol in ether solution was added to each petri dish to give a final concentration of 10mg/l MM. The plates were left overnight to allow the ether to evaporate and were subsequently innoculated with 4mm discs of innoculum taken from 5 day old colonies on SMA. Cultures comprising one experiment were randomised together and incubated for three weeks.

For counting, the petri dish base was inverted on the microscope stage and viewed under the low power objective. A plane of focus just inside the glass was selected and the petri dish was traversed from edge to edge, passing through the innoculum, continually adjusting the fine focus knob through one half of a turn only. Three transects were counted from each culture and three cultures were counted for each isolate.

Part VIII Asexual selection

Each generation of selection via zoospore propogation consisted of selecting the appropriate extreme phenotype from a family of 20 individuals and

proceeding to the next generation using the selected individual as parent. From the first generation the zoospore with the fastest growth rate was selected to establish the fast line, and the one with the slowest growth rate was selected to establish the slow line.

The growth rates of the twenty individuals of each family were determined in duplicate and the 40 petri dishes that this necessitated were randomised together during incubation. In all generations subsequent to the first, the petri dishes for determining the growth rates of fast and slow families were prepared and measured as one experiment and were randomised together during incubation.

Part IX Sexual selection

In all but one respect, selection from oospore progenies was by the same procedure as that used in the asexual selection experiment. Where the extreme individual of a family was infertile, the next fastest (or slowest) fertile oospore culture was selected.

Part X Statistical analysis

The results were analysed primarily by the use of analysis of variance and regression techniques (Snedecor and Cochran,1967) in order to asses the

variation between and within progenies and also the response to selection.

For each progeny analysis of variance was used to detect and to estimate the variation between and within offspring. The growth rate of each individual was measured in two replicates, so that typically there were 40 measurements for each progeny. Hence the expected mean squares were :

ITEM	EXPECTED MEAN SQUARE
BETWEEN INDIVIDUALS	$\sigma^2_e + 2\sigma^2_b$
ERROR (BETWEEN REPLICATES)	σ^2_e

The coefficient of σ^2_b was two because the measurements on each individual were duplicated.

Frequently replicates were lost through contamination and thus estimated variances could not be calculated as above. The coefficient of σ^2_b was calculated as (Snedecor and Cochran,1967 p.289-291):

$$\text{coefficient of } \sigma^2_b = \frac{n-\left(1+\frac{(n-r)}{n}\right)}{N}$$

where n = the number of observations

r = the number of missing observations

N = the between individuals degrees of freedom.

Chapter 3. Asexual variation

Part I Introduction

The spontaneous variants that occasionally appear among asexual progenies of wild type isolates may result from a change in either cytoplasmic or nuclear genetic material. Those due to nuclear genes could conceivably be the products of (1) the segregation of the components of a heterokaryon, (2) spontaneous mutation or, (3) parasexual recombination (Pontecorvo, 1956). The role of the parasexual cycle in relation to asexual pathogenic variation in Verticillium has been discussed by Hastie (1970) and is probably an important mechanism of variation in other asexual haploid fungi too. In diploid fungi, that part of the parasexual cycle involving mitotic recombination could occur. When zoospores of a strain of P.cactorum heterozygous for a recessive methionine requirement were exposed to ultraviolet irradiation, some methionine requiring single zoospore colonies were present among the survivors (Elliott and MacIntyre, unpublished observations). These colonies were most probably the products of mitotic recombination. One can imagine that this process could be of considerable

importance to asexual populations of Phytophthora (e.g. the A¹ mating type of P.infestans in Britain). However it must not be assumed that single mating type populations cannot reproduce sexually as Reeves and Jackson (1972) have demonstrated that the A^2 mating type of P.cinnamomi can form selfed oospores in certain soil conditions.

Many asexual variants exhibit non-Mendelian inheritance and can therefore be considered to be the result of cytoplasmic (extrachromosomal) variation. Jinks (1963) lists seven criteria on which cytoplasmic variation may be distinguished from nuclear variation but not always are all seven tests applicable to a single species. In P.cactorum neither reciprocal crosses nor a heterokaryon test are possible. Fincham and Day (1971) in their review of the subject, recognise discontinuous and continuous cytoplasmic variation as distinct from each other and cite the petito yeasts (Ephrussi,1953;Linnane and Haslam,1970) and the A variant of Aspergillus glaucus (Sharpe,1958) as examples of discontinuous variation. The petites are stable variants having a respiratory defect, while in Aspergillus, A differs from the wild type in a number of characters including growth rate. In contrast to the petites it continually segregates for wild type on ascospore and conidial propogation.

Discontinuous morphological variation within zoospore progenies, possibly cytoplasmic in origin, was observed for P.cactorum by Stamps (1953). Later, Buddenhagen (1958) found treatment with ultraviolet irradiation could greatly increase the frequency with which the variants arose. Shaw and Elliott (1968) also observed spontaneous and induced morphological variation in P.cactorum. As asexual progenies of some strains persistantly segregated they concluded that morphological variation in their strain was largely cytoplasmic in origin.

Examples of continuous cytoplasmic variation are very interesting because of the bearing the results may have on the interpretation of the process involved in the differentiation of the fungal thallus. For instance, samples of conidial progenies of Aspergillus glaucus form a continuous range of phenotypes differing in such characters as growth rate and fertility. When selection is applied, the response is gradual and positive. In the same species, successive conidial transfers are accompanied by a gradual decline in fertility, but so long as the line remains fertile, fertility can be restored to normal by a few single ascospore transfers (Jinks,1954). Similarly, repeated conidial transfers of Neurospora crassa result in a gradual decline in fertility (Ikoh and Monishita,1971). Two further examples of continuous cytoplasmic

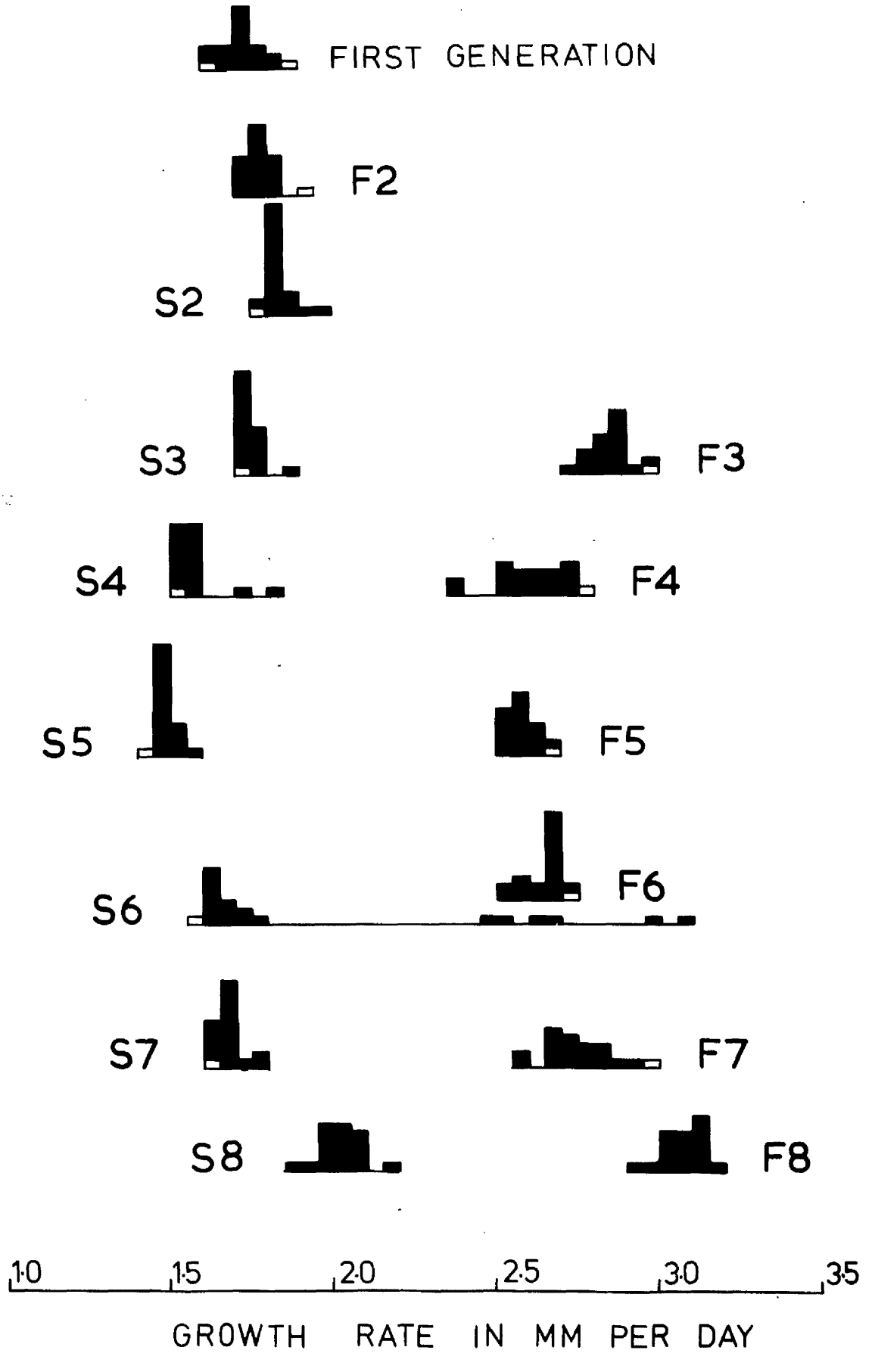
variation (for growth rate) in (i) the Basidiomycete Collybia velutipes (Croft and Simchen,1965) and in (ii) the Oomycete Phytophthora infestans illustrate that the phenomenon is possibly of widespread occurance in the fungi. Zoospore progenies of P.infestans show variation for growth rate in culture (Caten and Jinks,1968;Upshall,1969(a)) and for growth rate on host tissue (Caten,1970). Furthermore, the variation- which is continuous, heritable and probably cytoplasmic in origin - has been equated with the changes that occur during attenuation of stock cultures (Caten,1971).

Thus the aim of the asexual selection experiments in P.cactorum is to ascertain the nature and mechanism of asexual variation in wild type strains, if any, and consequently to determine whether the strains used in the oospore selection experiments are heteroplasmic or not.

Part II Results

The results of eight generations of selection for fast and slow growth rate among zoospore progenies of strain P94 are illustrated in Fig.4. Each unit area represents the mean of two independent determinations on each of the 20 individuals that comprised each family. The individual growth rate

FIG.4. Frequency distribution of the results of selection for fast and slow growth rate via zoospore propogation. The individuals selected as parents for subsequent generations are unblocked.


FIRST GENERATION
F2
S2
S3
F3
S4
F4
S5
F5
S6
F6
S7
F7
S8
F8
1·0
1·5
2·0
2·5
3·0
3·5
GROWTH RATE IN MM PER DAY

FAMILY	ERROR df.	ERROR MS	BETWEEN ZOOSPORES df	BETWEEN ZOOSPORES MS
First generation	20	0.0018	19	0.0101***
F2	20	0.0101	19	0.0042
F3	20	0.0054	19	0.0068
F4	20	0.0069	19	0.0271**
F5	20	0.0051	19	0.0063
F6	18	0.0025	19	0.0067*
F7	17	0.0185	19	0.0200
F8	16	0.0054	19	0.0058
S2	17	0.0011	19	0.0022
S3	20	0.0025	19	0.0027
S4	17	0.0042	19	0.0108
S5	15	0.0017	19	0.0021
S6	18	0.0186	19	0.4787***
S7	15	0.0011	19	0.0024
S8	15	0.0088	19	0.0075

Table 2. Analysis of variance of each generation of the fast and slow asexual selection lines.

* 5% Significance of the
** 1%
*** 0.1% between zoospore MS

determinations may be found in Appendix I.

Wild type colonies occasionally sectored for fast growth rate and it was from such a sector in one of the replicates of family F2 that innoculum was taken to initiate the next generation of the fast line. In subsequent generations the fast line maintained a growth rate approximately twice as fast as that of the slow line. Fast colonies never sectored as did some colonies of the slow line (e.g. families S3,S4 and S8). In only one family (S6) was there obvious evidence of segregation. In this instance the segregants were like the fast growing colonies of the fast line. They could have resulted from the segregation of determinants at zoospore formation or may have been due to the formation of a fast sector during preparation of the strip cultures.

The analyses of variance performed on the growth rate determinations obtained from each family are presented in table 2. The between progeny item compared with replicates was not significant for most families. However, for the first generation and family S6, it was significant at the 0.1% level. For F4 it was significant at 1% and for F6 and S4 it was significant at 5%.

Due to the quite large variation between generations (see Fig.4) that resulted from measuring the growth rates of each generation in a separate experiment, comparisons between families within selection lines were not possible. However, as the two

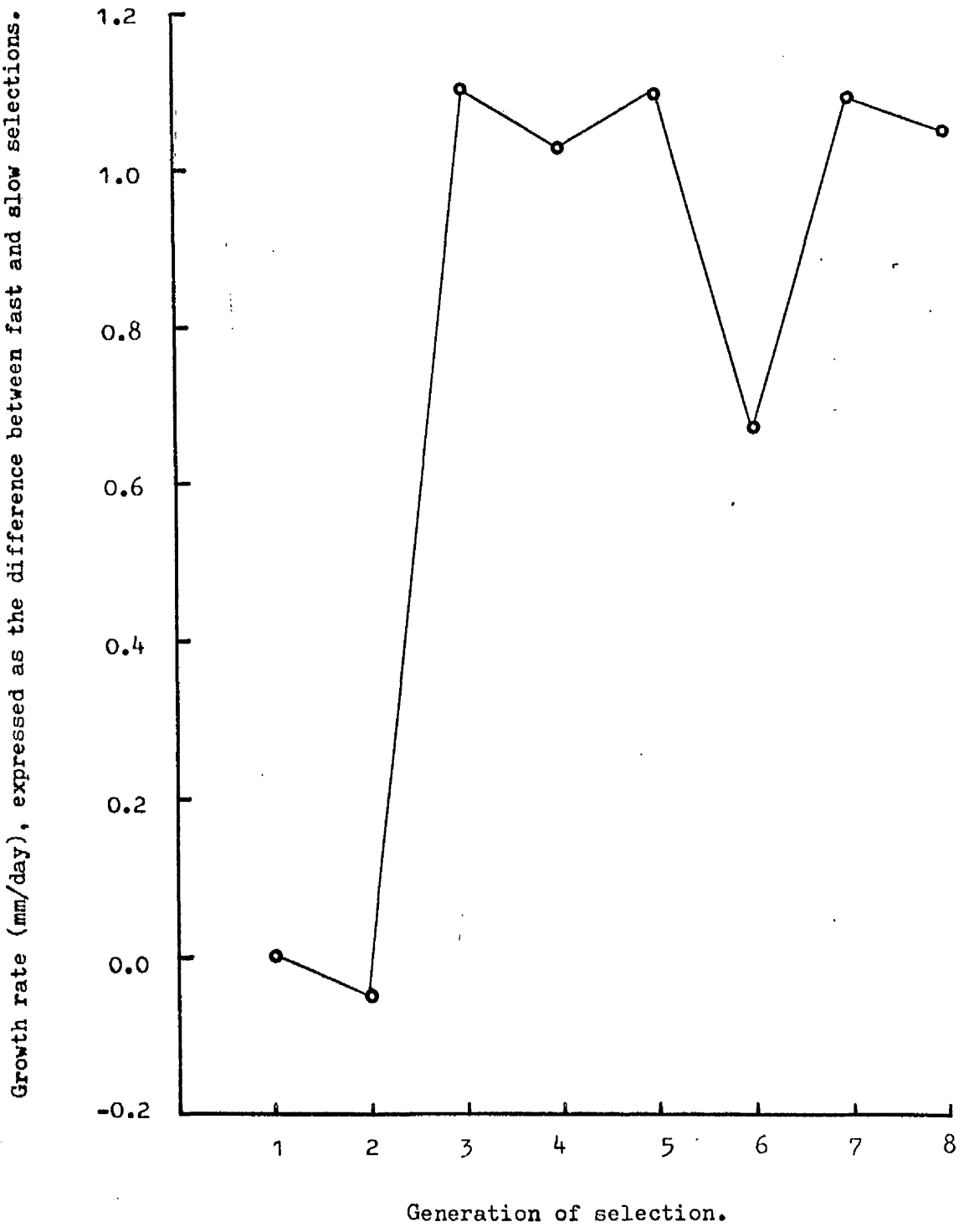


FIG.5, DIFFERENCE BETWEEN FAST AND SLOW ASEXUAL SELECTION LINES.

* For each generation the mean growth rate of the family from the slow line has been subtracted from that of the corresponding generation of the fast line.

families of each generation were measured simultaneously, this source of variation can be greatly reduced by determining the difference between fast and slow family means of each generation. These differences have been plotted in Fig.5. Generation 6 differed from the others as it was the generation in which segregation for fast growth rate occurred. Generations 1 and 2 preceeded the establishment of the fast growing sector of the fast line. For the remaining generations, 3, 4, 5, 7 and 8, the difference between fast and slow family means does not increase with generation and there is therefore no evidence for a response to selection.

The two selected zoospore parents of each generation, i.e. the fastest of the fast family and the slowest of the slow family, were stored in one ounce bottles on MM until the end of the selection experiment. From measurements of the growth rates of these isolates in one environment it would be possible to determine the nature of any changes in growth rate that occurred during the course of selection. Therefore innoculum was taken from the one ounce bottles and five growth rate determinations performed on each isolate (Fig.6). As there had been no overall response to selection apart from the establishment of the fast isolate, it was necessary only to analyse the growth rates obtained from isolates of the slow line.

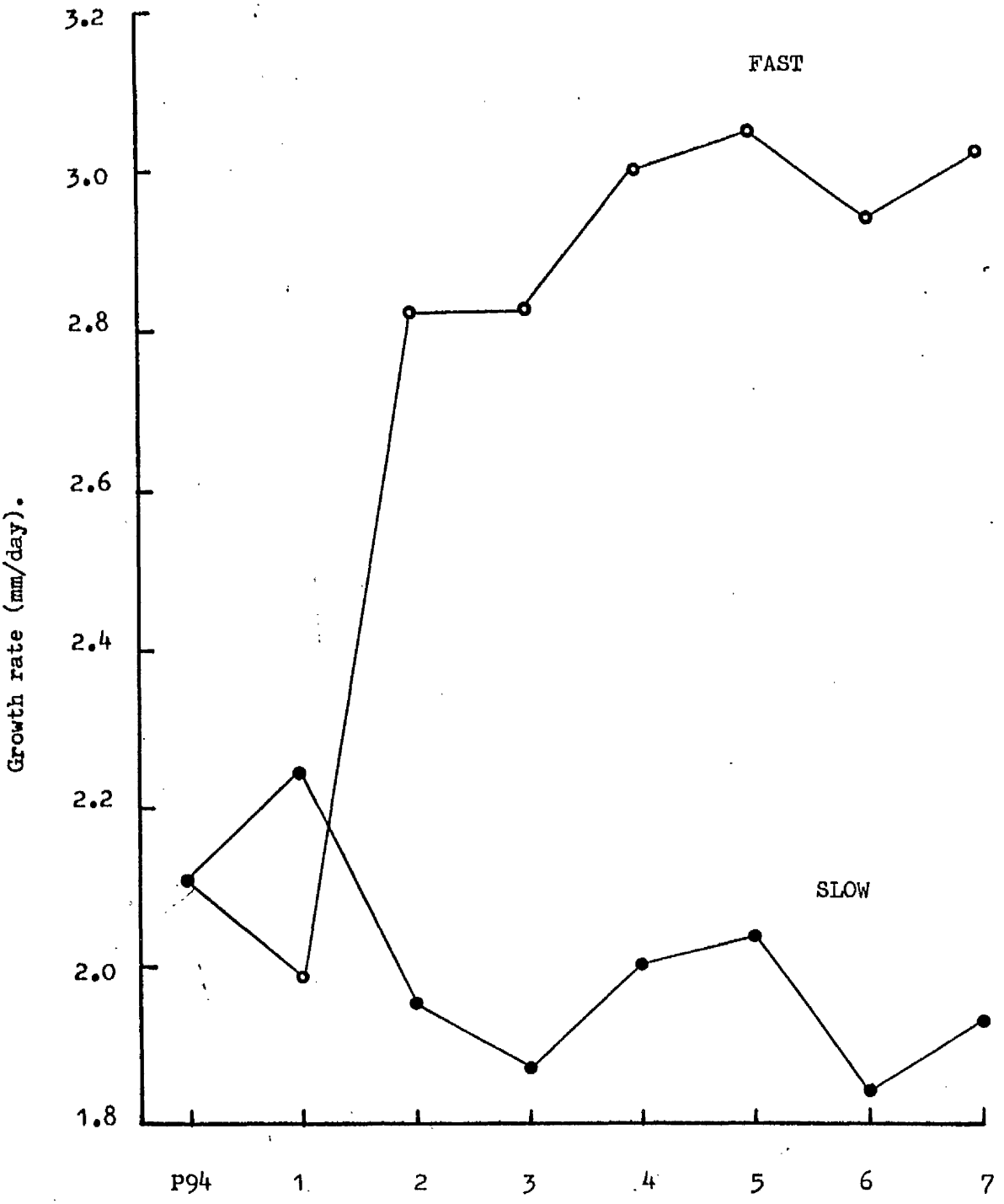


FIG.6, GROWTH RATES OF WILD TYPE P94 AND THE FAST AND SLOW SELECTIONS OF THE ASEXUAL SELECTION LINES.

ITEM	SS	df	MS	VARIANCE RATIO
Between Isolates				
Regression	0.24508	1	0.24508	4.13 not sig.
Remainder	0.35600	6	0.05933	5.15 sig.at 0.1%
Replicates	0.36892	32	0.01153	

The regression MS of growth rate against generation of selection is not significant compared with the remainder MS. This would indicate that the slow line retained a wild type growth rate throughout the course of selection and also that the fast line remained uniformly fast after the second generation. There were however significant differences between isolates when compared with the replicates MS. This probably reflects changes that occurred during storage of the selections.

Fertility of the wild type and the parents selected from generations 2,4,6 and 8 was determined by oospore counts. The counts obtained are presented in Table 3 and illustrated in Fig.7. To reduce the ~~error~~ variance in larger counts, individual counts were converted to $(x + \frac{1}{8})^{\frac{1}{2}}$ for the analysis of variance (Elliott,1968). Also, for comparison, counts were separated into those derived from isolates with a fast growth rate and those derived from isolates with a wild type growth rate. The analysis of variance of the counts is presented in Table 4. The mean

Family from which isolate was derived

P94	S2	S4	S6	S8	F2	F4	F6	F8
5	15	10	32	55	35	51	38	40
27	6	9	41	84	33	74	45	40
26	13	13	89	54	47	168	45	46
30	23	16	29	34	136	11	54	4
10	25	18	50	18	81	16	50	66
46	12	21	29	7	46	25	71	46
6	13	46	23	92	64	121	93	161
9	19	49	18	68	102	95	133	112
6	25	36	27	49	47	113	194	274

Table 3. Oospore counts for nine isolates derived from the asexual selection lines. For each isolate, three transects on each of three petri dishes were counted.

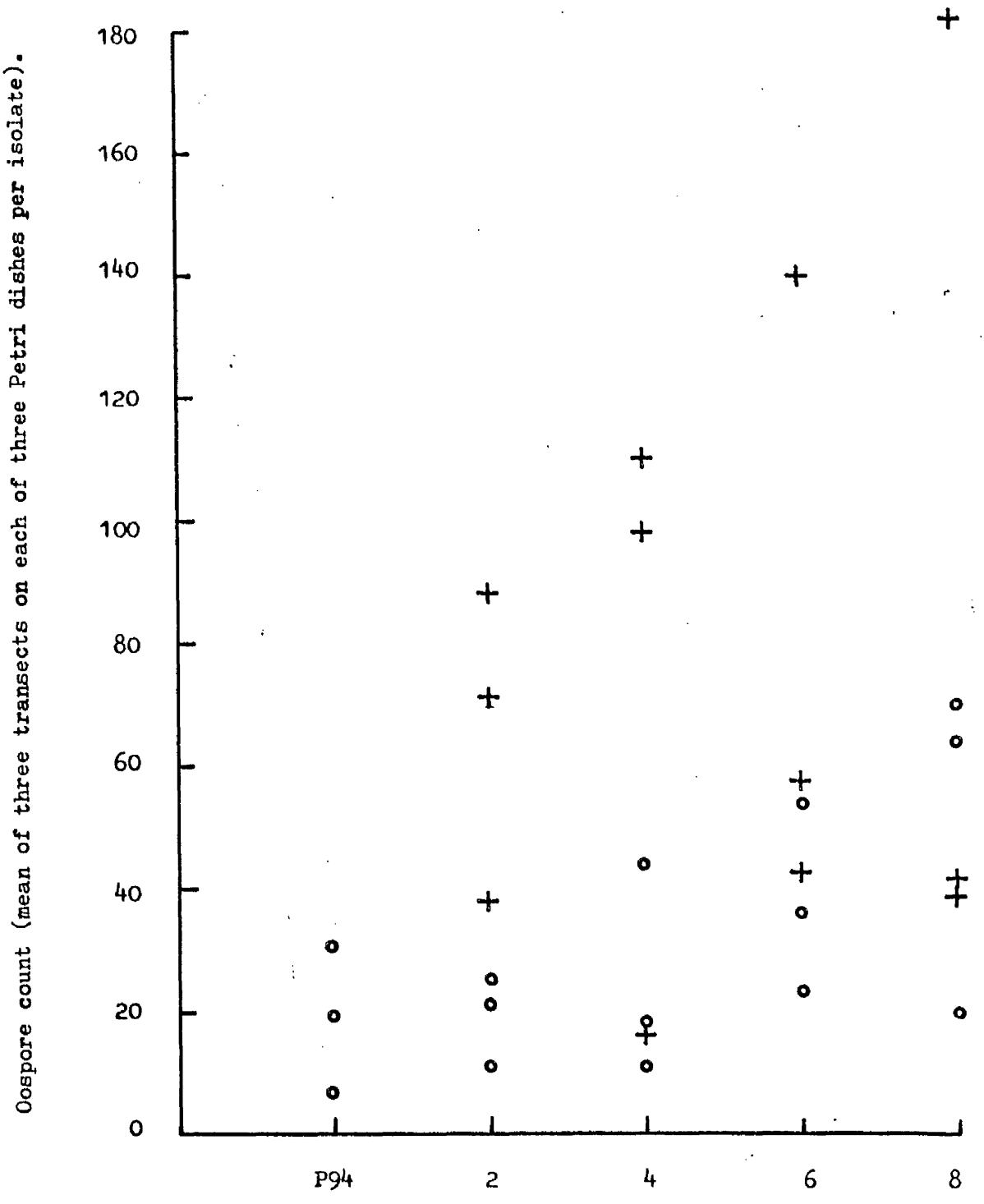


FIG. 7, OOSPORE COUNTS FOR NINE ISOLATES DERIVED FROM THE ASEXUAL SELECTIONS.

** Counts of the wild-type (P94), and the fastest isolates and the slowest isolates of generations 2, 4, 6 and 8 of the fast and slow lines respectively.

o Isolates with wild-type growth rate.

\+ Isolates with fast growth rate.

ITEM	SS	df	MS	VARIANCE RATIO
Fast isolates v Slow isolates	204.9847	1	204.9847	7.74 significant at 5%
Between fast isolates				
Regression	6.6688	1	6.6688	not significant
Remainder	0.2365	2	0.1183	
Between petri dishes	211.9556	8	26.4945	
Between counts	28.5648	24	1.1902	
Between slow isolates				
Regression	46.5894	1	46.5894	6.59 signf.at 5%
Remainder	3.8127	3	1.1709	
Between petri dishes	70.6877	10	7.0688	
Between counts	37.4981	30	1.2499	

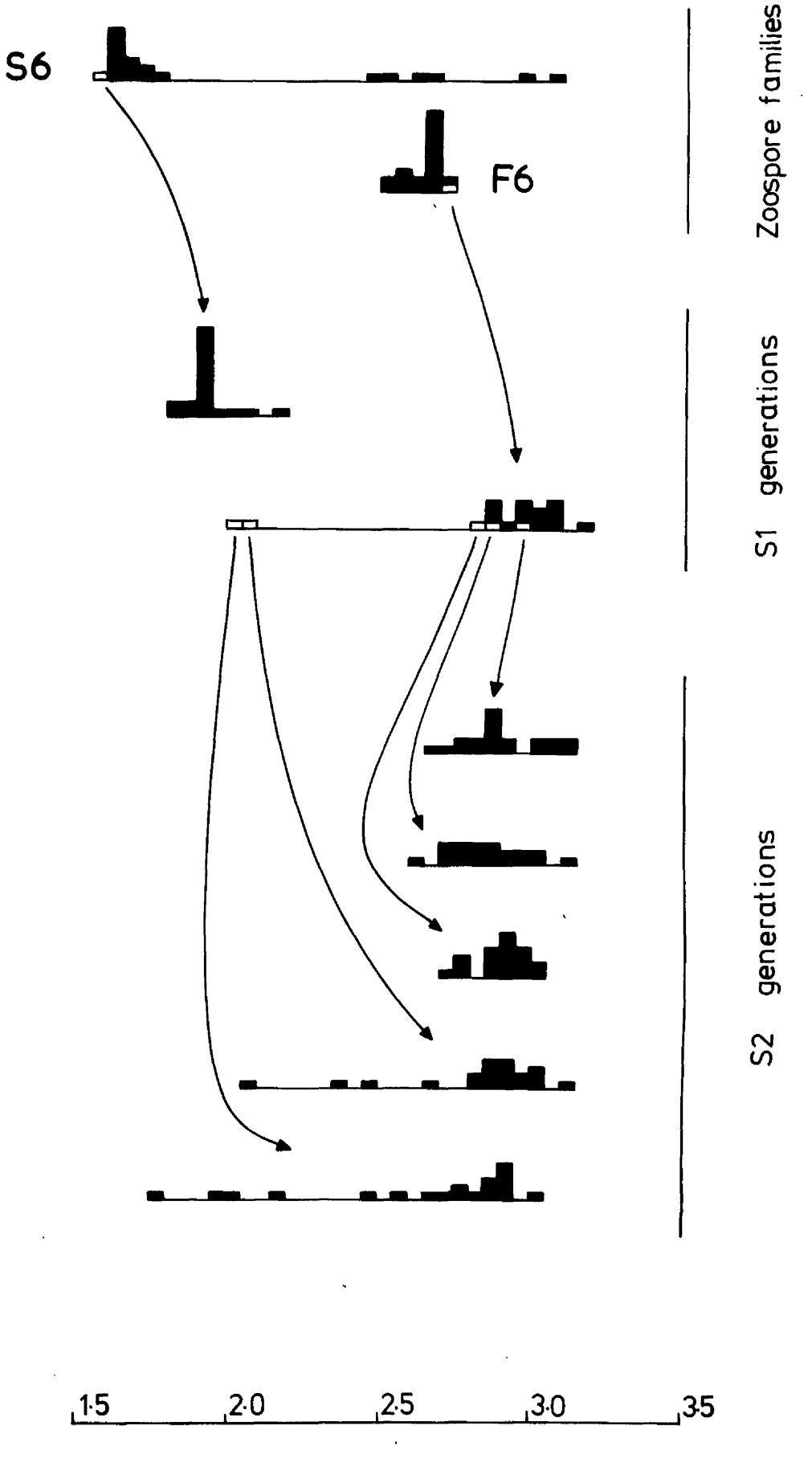
Table 4. Analysis of variance of the oospore counts of isolates obtained from the asexual selection experiment.

count for fast isolates was higher than that of the other isolates (significant at 5%). Also the counts for both appeared to increase with generation although the regression MS is significant (5%) only for isolates with a wild type growth rate.

As oospore progenies of P.cactorum are easily obtained, it is possible to perform a sexual analysis by selfing of the fast variant to determine whether its inheritance is Mendelian or not. Oospores were derived from the fastest zoospore colony of family F6 and, for comparison, the slowest zoospore colony of family S6. From the S1 family of the fast zoospore, five single oospore cultures were selected and taken to a further generation. The two S1 progenies and the five S2 progenies each consisted of 20 single oospore cultures, and on each a single growthrrate determination was made. These measurements are illustrated in Fig.8. The S1 offspring of the fast zoospore segregated 18 fast : 2 non-fast. Three of the fast oospore cultures gave uniformly fast S2 families, whereas the S2 families of the two non-fast oospore cultures continued to segregate in a similar fashion to the S1 family. The non-fast oospore colonies were unlike the wild type in that they invariably sectored for faster growth rate and after several mass hyphal transfers became typically fast.

Thus, the pattern of inheritance of the phenotype of the fast variant over two generations of selfing

FIG.8 Frequency distribution of S1 and S2 generation progenies of the fast growing variant.


S6
F6
Zoospore families
S1 generations
S2 generations
1·5
2·0
2·5
3·0
3·5
GROWTH RATE IN MM PER DAY

differed from the Mendelian inheritance expected of a single gene mutation in a diploid organism. If the fast phenotype was due to a dominant mutation, then the S1 generation would be expected to segregate 3 fast : 1 non- fast. Furthermore, S2 progenies of non-fast S1 individuals should be pure breeding and S2 progenies of S1 individuals showing the dominant phenotype should comprise both pure breeding and segregating families. The inheritance of the phenotype of the fast variant did not fit this expectation.

Furthermore, it may be argued that the fast variant resulted from a mitotic recombination. However the variant was phenotypically unlike any of the oospore cultures obtained from the wild type P94 (Fig.8 and Fig.9). It follows that the simplest explanation of the fast variant is one based on cytoplasmic determinants.

Part III Discussion of results

From an examination of the data on the variation between zoospores within families and the response to selection it is evident that zoospore propogation does not release continuous variation for growth rate. The highly significant variation between first generation zoospores (0.1%) and the loss of variation in subsequent generations is a situation comparable to

That observed by Boccas (1972) over two generations of zoospore propogation of P.syringae. Boccas suggested that greater variation in the first generation could have been due to the segregation of the components of a heterokaryon. This explanation is equally suitable for the results obtained from P94. It is possible that the strain was heterokaryotic when isolated or became so during the interval between isolation and the start of selection as a result of mutation of a process of nuclear segregation. If P94 was heterokaryotic, then one would expect families F2 and S2 to differ. In fact the difference between these two families is significant at 1%. However S2 had a

ITEM	SS	df	MS	VARIANCE RATIO
F2 v S2	0.04426	1	0.04426	7.43 sig.at 1%
Between zoospores	0.12155	38	0.00320	
Error	0.22040	37	0.00596	

faster mean growth rate than F2. As the estimated variance between zoospores in the first generation was quite small (δ^2_b = 0.0041) it is possible that selection was ineffective.

The oospore counts of the fast isolates were significantly greater than those of isolates with a wild type growth rate and for the slow selection line,

counts increased with progressive generations of selection. The increase in fertility over generations cannot be correlated with selection for growth rate as there was no response to selection apart from the establishment of the fast variant. Therefore if the change was in fact real it must have been due to some other cause. Elliott (1972) found that oospore counts gradually decreased when P.cactorum (strain IMI 21168) was maintained by repeated subculturing on MM over a period of 16 months, while the same strain stored in one ounce bottles on a complete medium remained fertile. An explanation compatible with both the results of Elliott and those of the selection experiments therefore cannot be one based merely on the state of growth of the fungus (active growth during repeated subculturing and quiescent growth during storage). Alternatively, changes in fertility could be dependant upon the nature of the medium on which the fungus is grown. In both instances a decline in fertility was associated with maintenance on MM, while the higher levels of fertility were recorded for strain IMI 21168 stored on a complete medium and strain P94 subcultured on a variety of media (PMA, PA, SMA and MM) during the course of the selection experiments.

The fast variant differed from the wild type in growth rate and fertility. It was phenotypically stable on zoospore propogation and inherited in a non-

Mendelian fashion through two generations of selfing. Some oospore progenies were uniformly fast, while others segregated into fast, and unstable non-fast single oospore cultures. In these respects it is interesting to compare the fast variant with some of the zoospore variants obtained by Shaw (1965). Among single zoospore cultures of a streptomycin dependant isolate of P.cactorum (strain IMI 21168) Shaw observed segregation for several distinct morphological types. One class of phenotype, which Shaw termed 'k' type, resembled the fast variant of P94 in not sectoring and having a growth rate approximately twice that of the wild type. It however persistantly segregated for k and wild type colonies on zoospore and oospore propogation. This led Shaw to conclude that k was probably cytoplasmically determined. He also obtained a streptomycin resistant (Sr) isolate, which in its pattern of inheritance more closely resembled the fast variant of P94. Sr bred true on zoospore propogation. Sexual offspring of Sr were largely Sr, but a few grew at first poorly on all concentrations of streptomycin, becoming typically Sr later. In the absence of known nuclear markers, it was not possible for Shaw to distinguish cytoplasmic from nuclear determination in this instance.

In previous studies on variation in Phytophthora, and particularly in P.infestans (Jeffrey, Jinks and Grindle, 1963; Paxman, 1963; Caten and Jinks, 1963; Upshall,

1969(a),(b);Caten,1970,1971), it has not been possible to distinguish between nuclear and cytoplasmic variation by performing a sexual analysis. In this study on P.cactorum, the analysis of selfed progenies provided a satisfactory means of distinguishing cytoplasmic from nuclear determination in the case of the fast variant. An explanation based on a single dominant mutation was discounted by comparing the observed pattern of inheritance with that expected for a diploid organism. Further, an explanation for the fast variant based on mitotic recombination was discounted by comparing the fast variant with the phenotypes of single oospore cultures derived from the wild type P94. Therefore as the inheritance of the fast phenotype is not typically Mendelian, the simplest explanation is one based on cytoplasmic determinants.

Chapter 4. Sexual Variation

Part I Introduction

Sexual progenies of wild type isolates of haploid fungi often exhibit variation for growth rate in culture. The individuals of a family usually differ from one another to a greater or lesser extent and combine to form a continuous, normal distribution for growth rate. Single ascospore cultures of Neurospora crassa (Papa,Srb and Federer,1966) and Aspergillus nidulans (Jinks,Caten,Simchen and Croft,1966) and single basidiospore cultures of Schizophyllum commune (Simchen,1966(a)) and Collybia velutipes (Croft and Simchen,1965) exhibit this kind of variation for growth rate. The sexual progenies of these four species were obtained from crosses between different wild type isolates, and the variation in growth rate was shown to result largely from the recombination of nuclear genes for which the parents differed. The continuous range of variation within one family resulted from the combined effects of several or more segregating loci. Therefore growth rate in these fungi is determined by a number of genes of similar effect,i.e. growth rate is under polygenic control. Contrasting with crossed progenies, the selfed progenies of haploid fungi are composed of individuals of uniform growth rate.

Ascospore cultures derived from homokaryotic mycelium of Aspergillus glaucus (Mather and Jinks,1958) or A.nidulans (Jinks,Caten,Sinchen and Croft,1966) by selfing do not exhibit variation for growth rate. The zygote in these instances results from the fusion of two identical gametic nuclei. Therefore all the ascospores of one family derived by selfing will be genetically the same. Alternatively, in a diploid fungus, selfing may result in genetic variation between offspring if the diploid nuclei of the parent are heterozygous at loci that determine growth rate. Genetic recombination will occur at meiosis and the selfed offspring will consequently exhibit variation. Boccas(1972) made use of these different expectations (i.e. that selfed progenies of a haploid organism will be uniform and that selfed progenies of a diploid organism may be variable) to obtain evidence to support a theory of diploidy for the homothallic species, P.syringae. From a single zoospore isolate of the species he obtained three oospore progenies and three zoospore progenies and determined their growth rates. In every instance the oospore progenies were more variable than the zoospore progenies. Presumably the isolate of P.syringae was diploid and heterozygous at loci controlling growth rate. Although the experiments performed by Boccas were rather limited, they were the first of their kind on Phytophthora and demonstrate

that it is possible to carry out a sexual analysis of quantitative variation in the genus.

From the analysis of sexual progenies of wild type isolates of a number of higher fungi, evidence of polygenic control of a variety of characters has emerged. Ascospore length in Neurospora crassa is a variable character which is largely determined by nuclear genes which exhibit linkage relationships (Pateman,1955,1959;Pateman and Lee,1960;Lee,1962;Lee and Pateman,1959,1961). Selection for fast and slow growth rate and crosses between selected lines has demonstrated that growth rate in N.crassa is under polygenic control (Papa,Srb and Fdderer,1966,1967;Papa, 1970,1971(a),(b)). Growth rate in Aspergillus nidulans is similarly determined (Jinks,Caten,Simchen and Croft, 1966;Butcher,1969). Polygenic control of the growth rate of monokaryotic mycelium has been demonstrated for the Basidiomycetes, Collybia velutipes (Croft and Simchen,1965) and Schizophyllum commune (Simchen,1966 (a);Conolly and Simchen,1968). In S.commune, selection for fast and slow growth rate resulted in a rapid response in both directions and in the fast line this was accompanied by a decline in the genetic variance. However, in the slow line, after the response to selection had ceased, there still remained considerable variation between cultures. This non-heritable variation was probably of cytoplasmic origin (Conolly and

Simchen,1968). The growth rate of dikaryotic mycelium and certain fruiting characters in Schizophyllum commune (Simchen and Jinks,1964;Simchen,1966(b),1967) and in Collybia velutipes (Croft and Simchen,1965) are also under polygenic control.

In higher plants and the examples cited above, non-nuclear effects usually contribute very little to the variation between sexually produced offspring. Of by far the greatest consequence are those effects due to nuclear genes. By performing the appropriate crosses it is possible to separate the genetic variance into components due to non-allelic interactions and dominance and additive effects. This has been done for the growth rate of Aspergillus nidulans (Jinks,Caten, Simchen and Croft,1966;Butcher,1969) and the growth rate of Neurospora crassa (Papa,1970). In both instances the growth rate variance was found to be due largely to non-allelic interactions although small additive effects were detected. There are unfortunately no comparable data for the growth rate of monokaryotic mycelium in either Schizophyllum or Collybia. Variation in the growth rate of dikaryons and in fruiting in S.commune (Simchen and Jinks,1964;Simchen,1966(b)) and in C.velutipes (Simchen,1965) was found to be due almost entirely to dominance and additive effects. In S.commune, small non-allelic interactions were detected for fruiting characters (Simchen,1966(b)) and the growth rate of dikaryons in crosses between strains

obtained from different populations (Simchen, 1967).

An understanding of the genetic mechanisms involved in the determination of quantitative traits is particularly important to the plant or animal breeder who seeks to improve economically desirable characters. However, animals and higher plants are technically and genetically less suitable for studies of quantitative genetics than are micro-organisms. Fripp and Caten (1971) pointed out that S.commune may prove very useful in deriving biometrical priniples applicable to diploid organisms, particularly higher plants. In S.commune the associated pair of haploid nuclei in each cell of the dikaryotic mycelium appear to determine the phenotype in a way similar to that expected of diploid nuclei. Although the genetics of Phytophthora are less well understood than the genetics of Schizphyllum, the vegetative mycelium of Phytophthora is diploid. Therefore studies of quantitative genetics in Phytophthora may yield biometrical principles of more general application to diploid organisms than could be expected to result from studies on Schizophyllum.

Sexual variation in strain P94 of P.cactorum has been studied by selecting for fast and slow growth rate over seven generations of oospore propogation. Limited studies of sexual variation in strain P205 of P.cactorum have also been carried out. As the degree of inbreeding in the sexual selection experiments is high,

the variation between oospore cultures within families is expected to decrease with progressive generations of selection. If (i) there are no cytoplasmic effects, (ii) the genes controlling growth rate are unlinked and (iii) all genotypes are equally viable, then the level of heterozygosity will decrease by half for each generation of selfing. Consequently the genetic variance will decrease by half for each generation and the response to selection will be gradual and will decline towards an upper limit as complete homozygosity is approached (East and Jones,1919;Lerner,1958). Even if the three conditions mentioned above are not fulfilled, the level of heterozygosity cannot reasonably be expected to increase during inbreeding. Failure of any of these conditions may influence the results obtained from a selection experiment. Cytoplasmic effects may result in greater variation between individuals within families. During selection for slow growth rate in Schizophyllum commune a high genetic variance was maintained without a response to selection. Non-heritable variation in this instance was thought to be due to cytoplasmic effects (Simchen,1966(a);Conolly and Simchen,1968). Close linkage between heterozygous loci controlling growth rate may conserve genetic variability until such linkages are broken. These linkages will be broken infrequently and during the course of selection will result in sporadic increases in the genetic variance (Sismandis,1942;Mather and Harrison,1949;

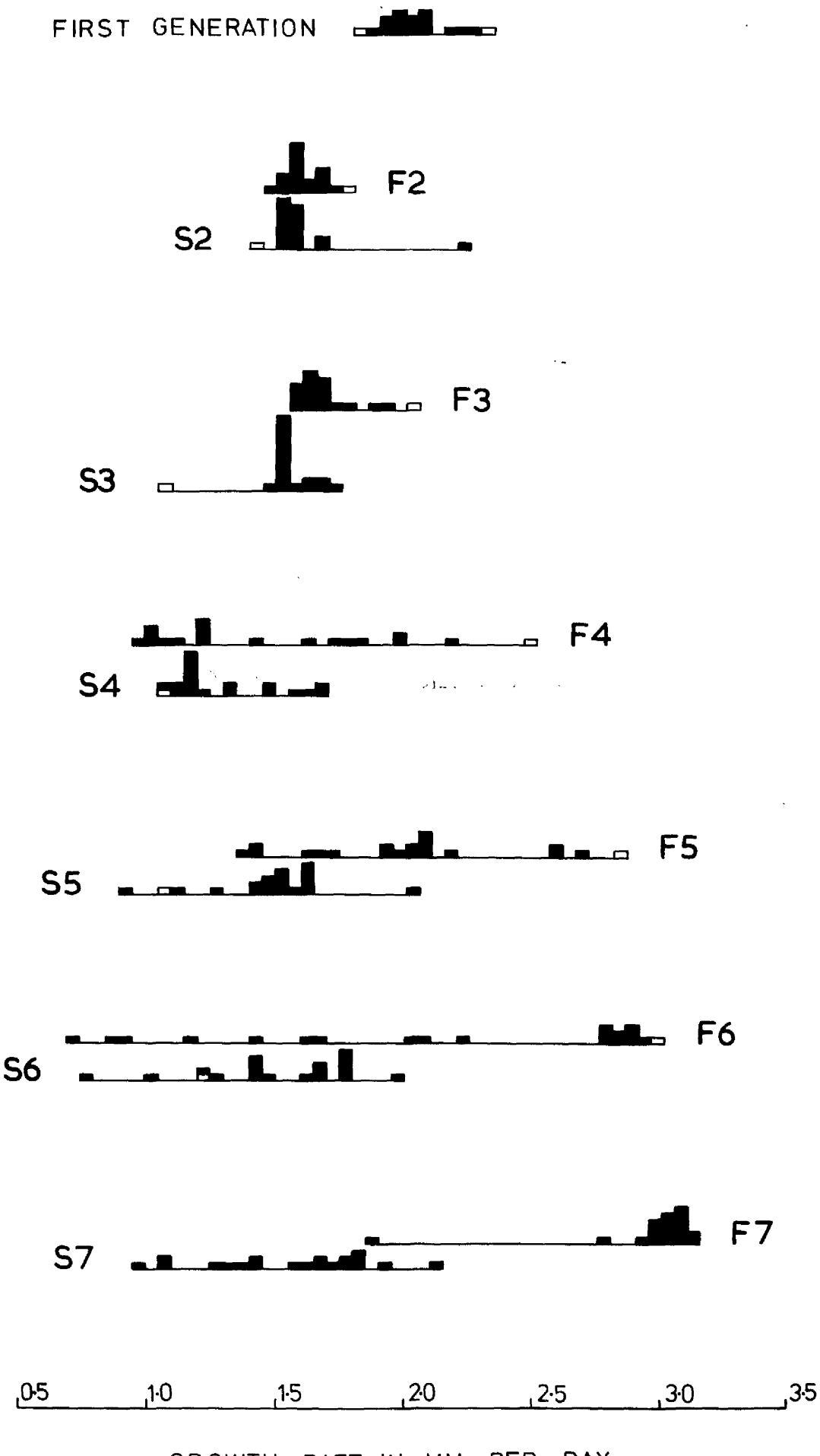
Papa,Srb and Federer,1966). Differential viability may result in only those individuals posessing certain genotypes reaching maturity and therefore being subjected to selection. In particular, heterozygotes may preferentially survive to maturity (Lerner,1954). Homozygotes often exhibit features of inbreeding depression such as deterioration of characters associated with fitness, i.e. characters associated with reproductive capacity. The superior viability of heterozygotes could result in the conservation of genetic variability during the course of inbreeding and selection. A further consequence of inbreeding depression may be and increase in the phenotypic variance resulting from poor canalisation of development. However such variation is of environmental origin and may be detected as the difference between clonal isolates (Lerner,1954).

The effect of inbreeding depression upon fertility has been investigated by performing oospore counts on representative isolates of the sexual selection experiment on strain P94. Fertility is a fitness character and may be expected to deteriorate during inbreeding and selection.

Part II Results of selection for growth rate in strain P94

Seven generations of sexual selection for fast and slow growth rate of strain P94 were carried out.

FIG.9 Frequency distribution of the results of selection for fast and slow growth rate via oospore propogation. The individuals selected as parents for subsequent generations are unblocked.


FIRST GENERATION
F2
S2
F3
S3
F4
S4
F5
S5
F6
S6
F7
S7
0·5
1·0
1·5
2·0
2·5
3·0
3·5
GROWTH RATE IN MM PER DAY

Each family consisted of twenty single oospore cultures on which duplicate growth rate determinations were made (Appendix II). The growth rate measurements obtained are illustrated in Fig.9. Each unit area represents the mean value of the two replicate determinations for each individual. From all families except S5 and S6, the fastest (or slowest) single oospore culture was selected as the parent for the next generation of the line. Due to increasing infertility in the slow line, the second slowest oospore culture was selected from family S5 and the third slowest was selected from family S6.

Comparing Fig.4. for zoospore progenies with Fig.9 for oospore progenies, it is clear that oospore propogation releases greater variation than zoospore propogation. From the analysis of variance of each family of the sexual selection experiment it was found that the variation between oospores was highly significant (0.1%) in every instance (table 5.). The comparable analysis of zoospore families (table 2.) detected variation between zoospores at this level of significance on only two occasions.

During the investigation of asexual variation in strain P94 (Chapter 3.) evidence was obtained that indicated that the wild type isolate of strain P94 was heterokaryotic for growth rate. Significant variation was detected between first generation zoospore cultures and second generation zoospore

FAMILY	ERROR df	ERROR MS	BETWEEN OOSPORES df	BETWEEN OOSPORES MS
First generation	19	0.0072	19	0.0336***
F2	19	0.0016	19	0.0098***
F3	14	0.0018	19	0.0211***
F4	19	0.0107	18	0.4072***
F5	19	0.0515	19	0.3120***
F6	19	0.0175	19	1.2303***
F7	20	0.0075	19	0.1537***
S2	19	0.0046	18	0.0581***
S3	18	0.0002	19	0.0286***
S4	18	0.0044	19	0.0776***
S5	19	0.0009	19	0.1105***
S6	20	0.0042	19	0.1746***
S7	19	0.0124	19	0.2950***

Table 5. Analysis of variance of the families of the sexual selection experiment.

*** 0.1% level of significance

families differed in mean growth rate. If the wild type of P94 was heterokaryotic then the variation between first generation oospore cultures would result from the combined effects of recombination between and within the components of the heterokaryon. The estimated variance between first generation oospore cultures was 0.0135 and the estimated variance between first generation zoospore cultures was 0.0041. The greater variation between oospore cultures than between zoospore cultures indicates that at least some of the nuclei of the wild type isolate of P94 were heterozygous for growth rate. To test whether or not nuclei of the wild type were heterozygous , twenty single oospore cultures were obtained from a single zoospore isolate of P94 and their growth rates determined in duplicate. The analysis of variance of the growth rates detected significant variation between oospore cultures at the 2% level.

ITEM	SS	df	MS	VARIANCE RATIO
Between Oospores	0.2273	19	0.0120	1.61 significant at 2%
Error	0.1482	20	0.0074	

The estimated variance between oospore cultures was 0.0023. As expected this value is smaller than the estimated variance obtained from oospore cultures derived directly from the wild type isolate of strain P94. Therefore the wild type isolate of P94 was probably heterokaryotic and at least some of the component

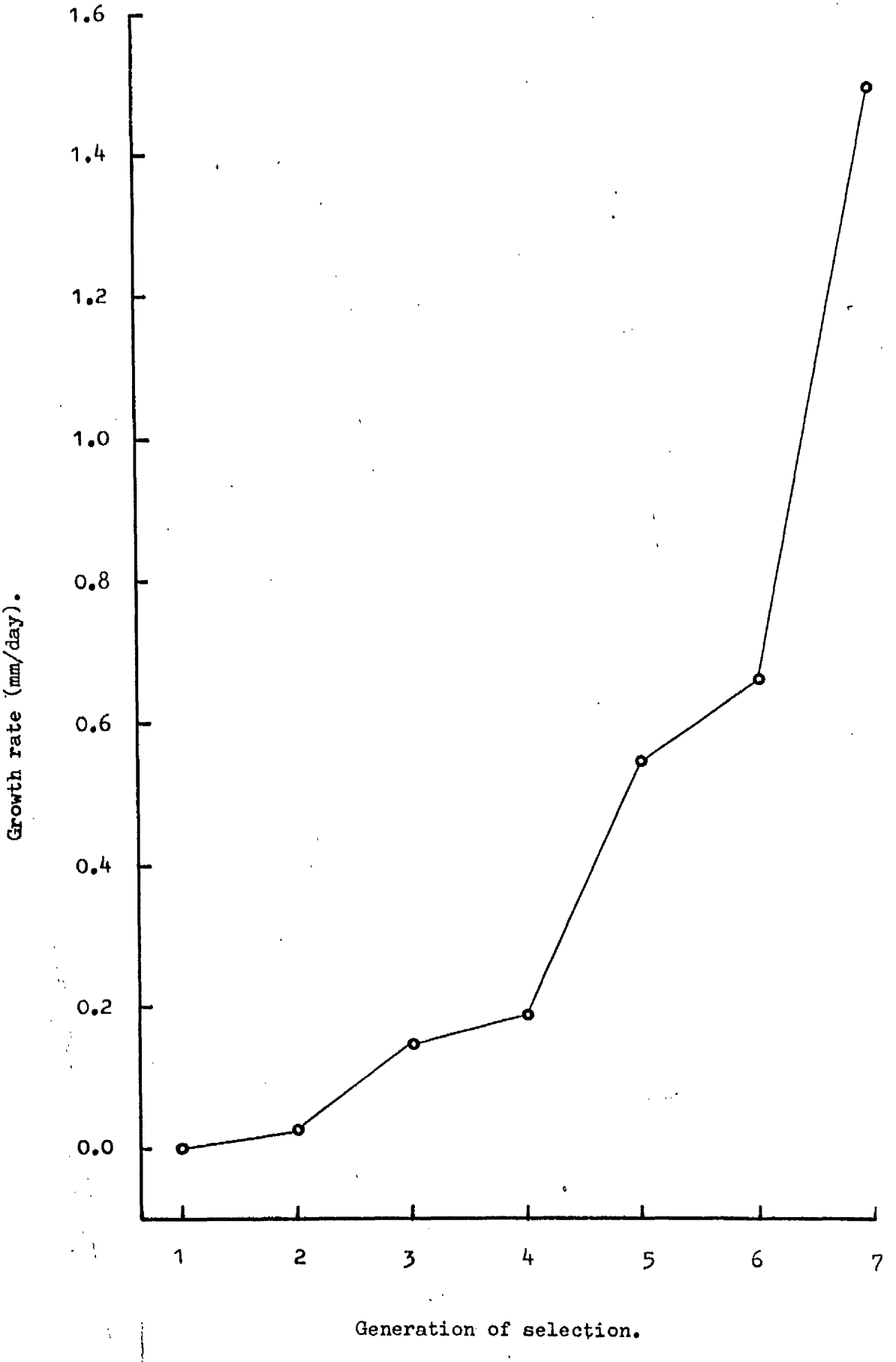


FIG.10, DIFFERENCE IN MEAN GROWTH RATE OF FAST AND SLOW FAMILIES OF EACH GENERATION OF THE SEXUAL SELECTION LINES.

nuclei were heterozygous at loci that determine growth rate. Heterkaryosis is unlikely to be a source of variation in generations of selection subsequent to the first generation as the oospores of P.cactorum are uninucleate (Blackwell,1943(a)).

If for each generation of both selection lines there exists significant variation between oospore cultures, then the response to selection should be gradual and positive in both directions. There was however considerable environmental variation between generations and this obscured the response to selection in individual lines. As fast and slow families of each generation were measured in one experiment, the response can be illustrated in terms of the difference between fast and slow family means. The determination of the total response in this way greatly reduces the environmental variation between generations of selection (Falconer,1960,p198-200). The total response when plotted against the generation of selection was found to increase gradually (Fig.10.). The total response was initially small but in later generations of selection it was large.

To determine whether or not a response had occurred in both selection lines the growth rates of cultures selected from each family and used as parents for the next generation were determined in one experiment. The parents had been stored in one ounce bottles on MM since the time of their isolation. Innoculum was

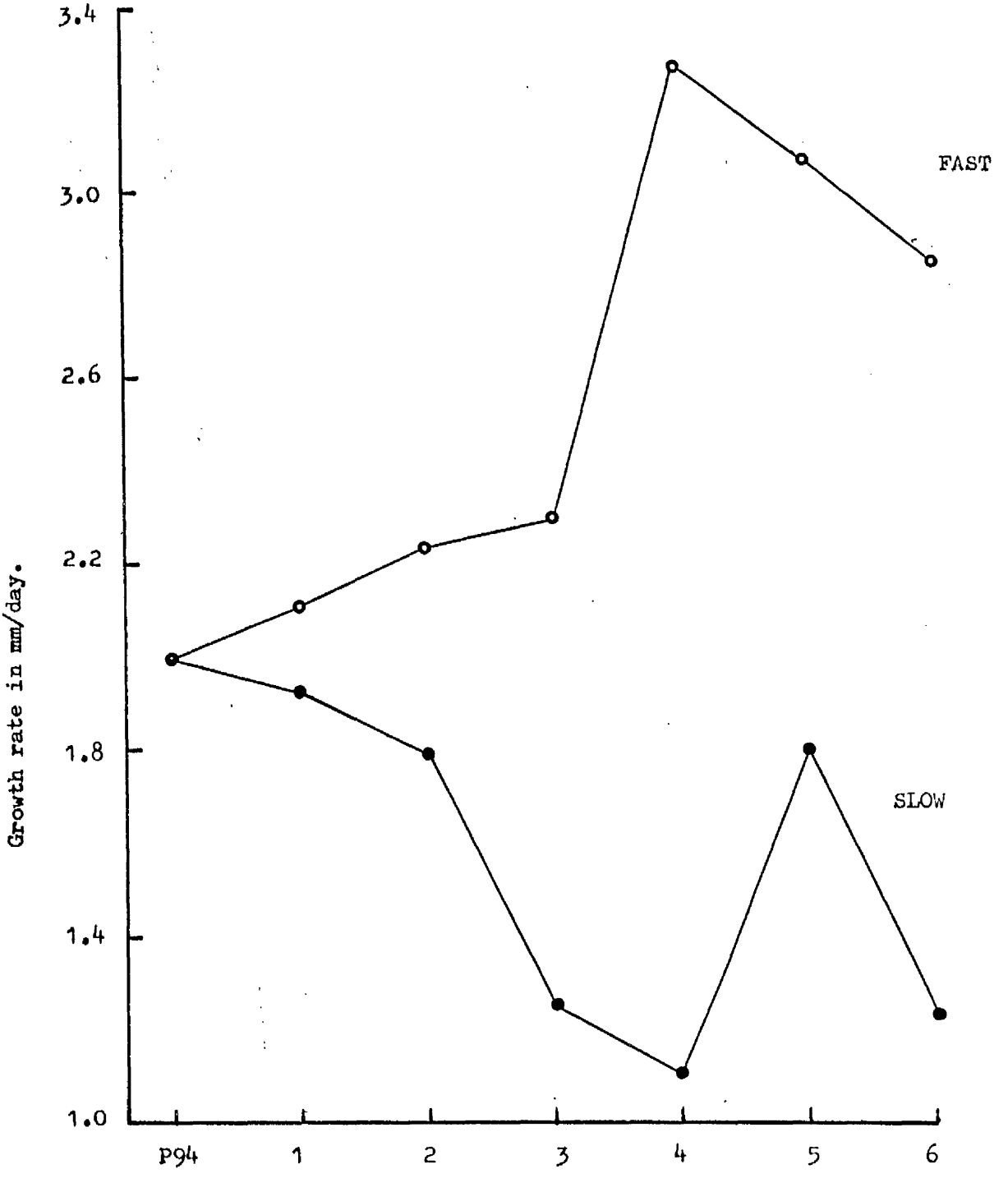


FIG.11, GROWTH RATES OF WILD TYPE P94 AND THE FAST AND SLOW SELECTIONS OF THE SEXUAL SELECTION LINES.

removed from the bottles and five replicate growth rate determinations obtained for each parent. In Fig.11. the mean growth rate of each parent has been plotted against the generation of selection from which it was obtained. The response in terms of parental growth rates was observed to be positive for both the fast and slow selection lines. This was particularly evident if only those parents selected from generations 1,2,3,and 4 were considered. The mean growth rates of the parents selected from families F5 and F6 were lower than might have been expected and those of the parents selected from families S5 and S6 were higher than expected. It is possible that the growth rates of these four parents had altered during storage in one ounce bottles.

From a plot of the overall response against the accumulated selection differential, the realised heritability of the selection experiment was calculated (Falconer,1960,p.202-203). The selection differential is the difference between the growth rate of the individual selected as the parent for the next generation and the mean growth rate of the family of which it was a member. The accumulated selection differential is the sum of selection differentials for all the generations. The response is the increase (or decrease) in the family mean with increasing generations of selection. The realised heritability

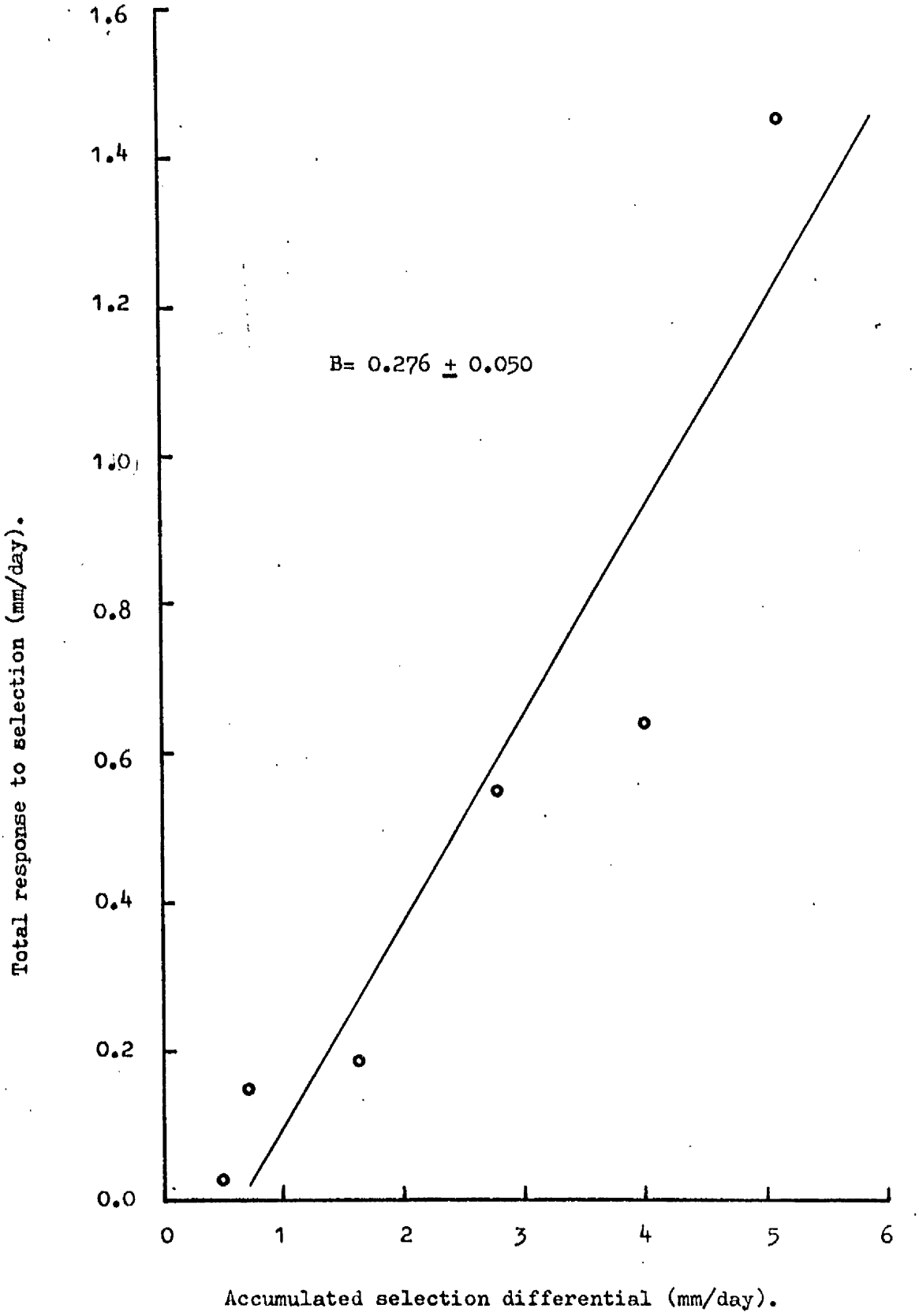


FIG.12, MEAN REALISED HERITABILITY OF THE FAST AND SLOW SELECTION LINES.

is equal to the regression coefficient of the response on the accumulated selection differential. If the mean growth rate of each family is identical to its parents growth rate then the character is completely heritable and the curve will be linear with a slope of one. To reduce the environmental variation between generations, the response and the accumulated selection differential was summed for fast and slow families of each generation (Simchen,1966 (a)). The accumulated selection differential was plotted against the response for each generation and a realised heritability of 0.276 ± 0.050 obtained (Fig.12). This value is the mean of the fast and slow selection lines and indicates that overall, approximately 27.6 percent of the variation between oospore cultures was heritable, while the remainder was non-heritable.

As mentioned above, in every generation of selection the variation between oospore cultures was significant compared with replicates of the same cultures and thus there was genetic variation in the population. It is obvious from Fig.9 and Table 5 that the variation between oospore cultures within families increased with increasing generations of selection. From the growth rate data of each family, estimates of the variance between oospore cultures (σ^2_b) and the error variance (σ^2_e) were obtained. These values are presented in Table 6 and Fig.13. Throughout the course of selection σ^2_e remained small for both lines and did not alter

FAMILY	MEAN GROWTH RATE	σ^2_e	σ^2_b
First generation	2.037	0.0072	0.0135
F2	1.606	0.0016	0.0042
F3	1.685	0.0018	0.0114
F4	1.478	0.0107	0.1982
F5	2.012	0.0515	0.1337
F6	2.160	0.0175	0.6223
F7	2.961	0.0075	0.0731
S2	1.583	0.0046	0.0267
S3	1.538	0.0002	0.0149
S4	1.292	0.0044	0.0385
S5	1.464	0.0009	0.0562
S6	1.502	0.0042	0.0852
S7	1.472	0.0124	0.1450

Table 6. Estimates of the variance between oospore cultures (σ^2_b) and the error variance (σ^2_e) for each family of the sexual selection experiment.

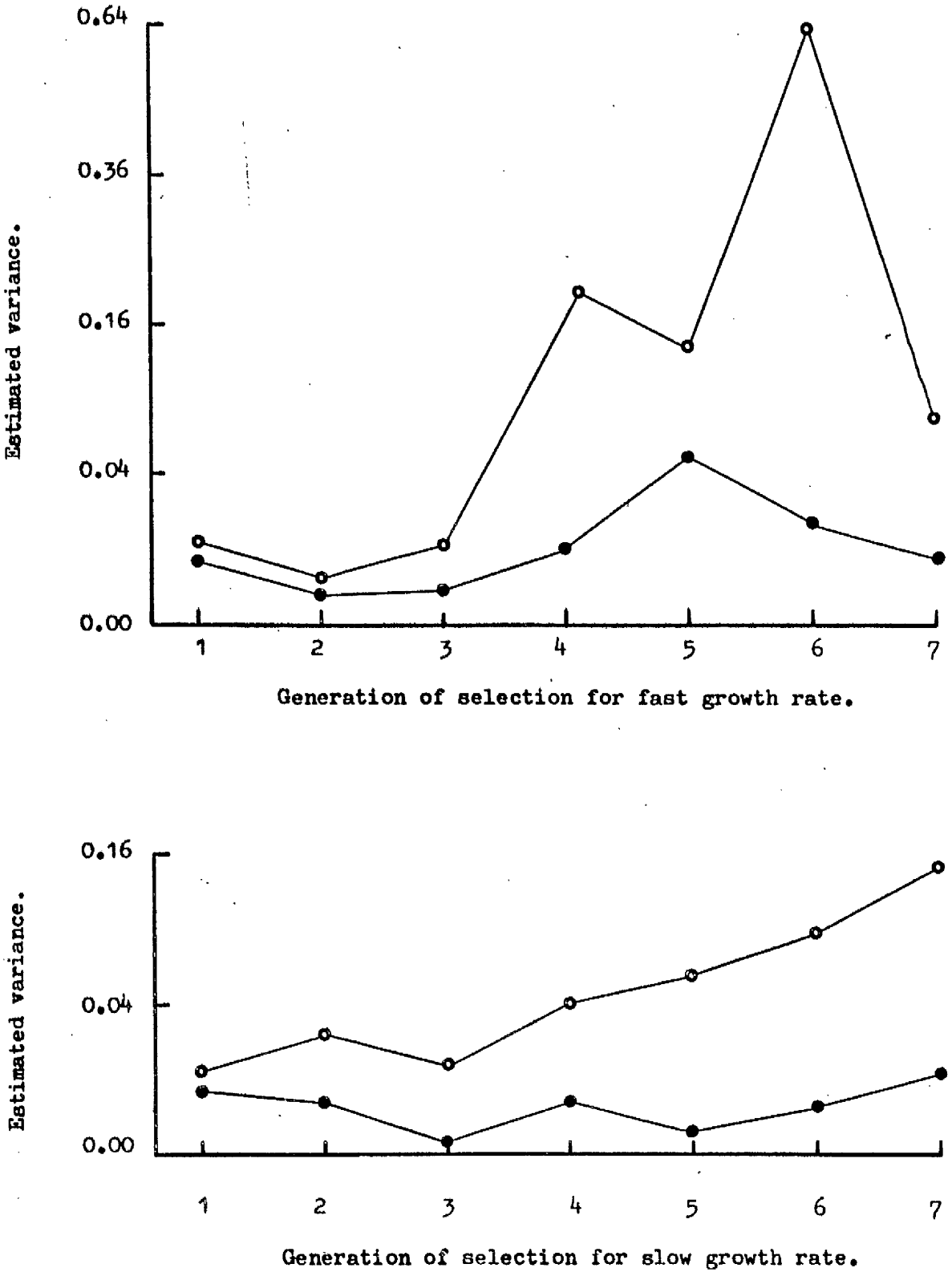


FIG. 13, THE CHANGES THAT OCCURED IN THE COMPONENTS OF VARIATION DURING THE COURSE OF SEXUAL SELECTION FOR FAST AND SLOW GROWTH RATE.

- ○ Estimated variance between oospores.
- ● Estimated error variance.

over generations. σ^2_b for families of the slow line increased gradually over the seven generations of selection. For the fast line σ^2_b increased rapidly over the first six generations of selection but from the seventh generation (family F7) a low value was obtained. This corresponds with the growth distribution of F7 illustrated in Fig.9. Most of the individual oospore cultures of family F7 had a fast growth rate; in fact they had the characters of the fast variant encountered during the asexual selection experiment (Fig.4).

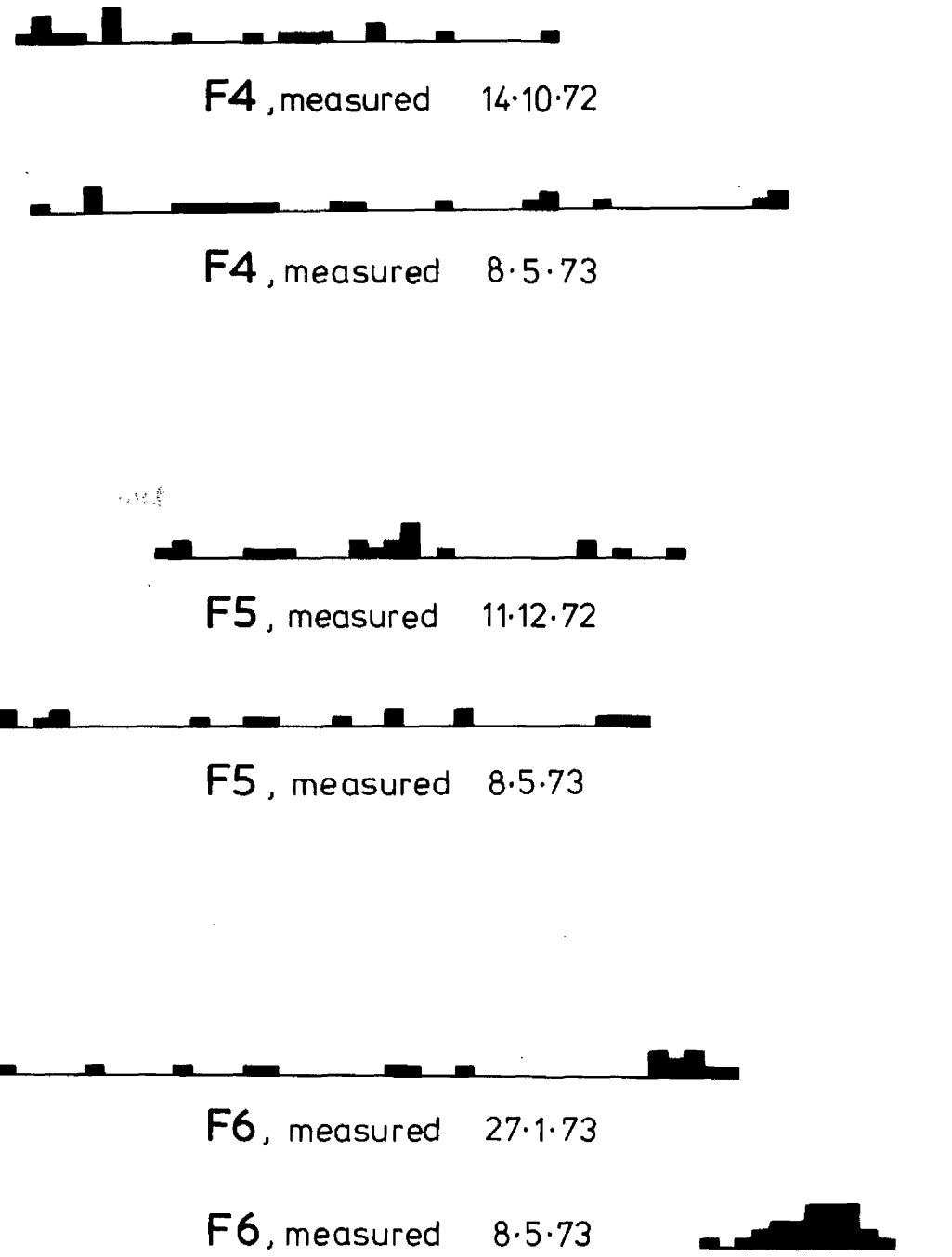
It has been argued in Chapter 3 that a change to a uniform fast growth rate is under cytoplasmic control. This view is borne out by a re-examination of oospore families F4,F5 and F6. Like F7 a proportion of the oospore cultures of the previous generation,F6, also had a growth rate similar to that of the cytoplasmic fast variant (see Fig.9). It is possible that in family F6 the offspring had segregated into fast and non-fast types and the selection of a fast culture, as the parent of the next generation, had resulted in family F7 being largely fast. Shaw and Elliott (1968) found that the pattern of segregation for a cytoplasmically determined morphological character in a strain of P.cactorum altered during storage of the strain. After storage at 3°C more morphological segregants appeared among zoospore progenies than were

obtained before storage. If segregation for a cytoplasmically determined fast growth rate had occurred in family F6 and possibly previous generations of the fast line, then when these oospore families are obtained for a second time from stored material their pattern of segregation might differ from that of the first occasion. Consequently, innoculum of the parents of families F4, F5, and F6 was taken from the one ounce bottles in which the isolates had been stored and a number of single oospore cultures obtained from each. Two growth rate determinations were performed on the individuals of each of the three families. The mean growth rates of the individuals of the three families obtained on the first and the second occasion are illustrated in Fig.14. The estimated variances between oospore cultures within families were :

	$\hat{\sigma}^2_b$	
FAMILY	FIRST OCCASION	SECOND OCCASION
F4	0.1982	0.5019
F5	0.1337	0.4132
F6	0.6223	0.0058

There was no marked difference in the growth rate variance of families F4 and F5 between the first and second occasion. However, family F6 which had a high variance on the first occasion, had a low variance on

FIG.14 Frequency distribution of oospore families F4,F5,F6 and oospore families obtained from the same parents at a later date.


F4, measured 14·10·72
F4, measured 8·5·73
F5, measured 11·12·72
F5, measured 8·5·73
F6, measured 27·1·73
F6, measured 8·5·73
0·5
1·0
1·5
2·0
2·5
3·0
3·5
GROWTH RATE IN MM PER DAY

the second occasion. The single oospore cultures of F6 obtained on the second occasion all had growth rates similar to that of the cytoplasmic fast variant. If the variation between oospore cultures of family F6 was due solely to the segregation of nuclear genes then all progenies obtained from the parent of this family should be similar. That the two progenies obtained differed so markedly suggests that the fast growth rate was cytoplasmically determined.

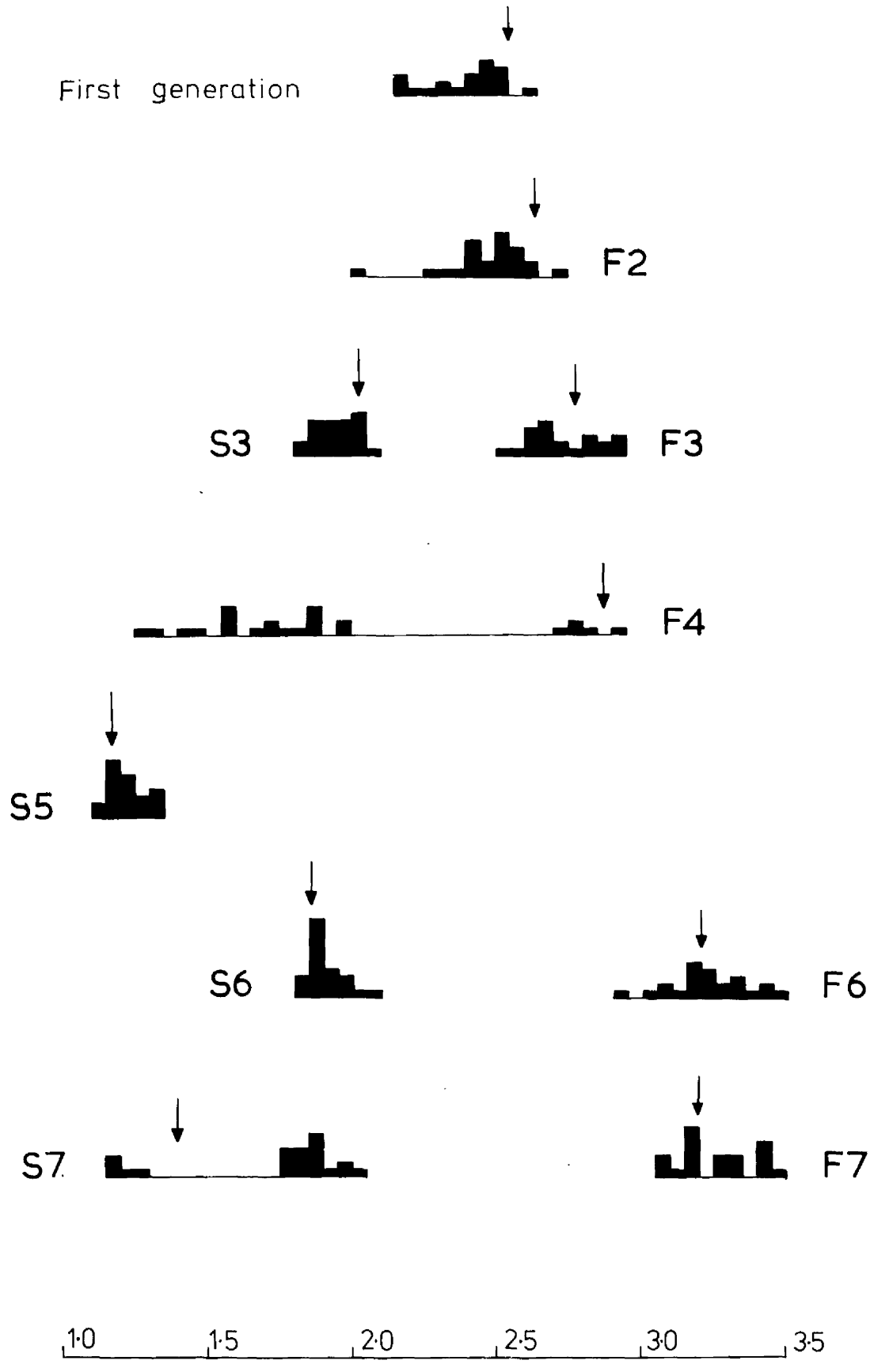
Segregation for a cytoplasmically determined fast growth rate will account for a greater part of the variation in family F6 and will explain the response to selection in family F7, but will not explain the behaviour of other families of the sexual selection experiment. In both lines the variance between oospore cultures within families increased with increasing generations of inbreeding and selection. This differs from the expected results of inbreeding and selection that have been discussed in the introduction to this chapter. In the simplest situation where variation results from independant recombination of nuclear genes the variability of progenies is expected to decrease by half for each generation of inbreeding. A departure from this expectation is not unusual as in many selection experiments the genetic variance has remained constant (Cooper,1959;Simchen,1966(a);Connolly and Simchen,1968) and in others the genetic variance has actually increased with selection and inbreeding (Falconer,1955;Robertson

and Reeve,1952;Clayton and Robertson,1957;Clayton, Morris and Robertson,1957). There are several factors that could result in an increase in variability during inbreeding. Poor canalisation due to in-breeding depression may result in greater variability of environmental origin (Lerner,1954). However this is an unlikely cause of the observed increase in variation as the estimated error variance remained constant throughout the course of selection. Increased variability may result from the breakage of tight linkages between loci heterozygous for growth rate. However such events are usually infrequent (Sismanidis,1942;Mather and Harrison,1949;Papa,1966) and therefore could not satisfactorily account for the observed increase in variability which was gradual and occurred in both selection lines. If however, the heterozygous loci in the wild type isolate of strain P94 differ in the magnitude of their effect upon growth rate, then linkage may account for the observed gradual increase in variability. Suppose that there are a small number of loci having major effects and a selective advantage as heterozygotes and that these loci are linked to a large number of loci with small but opposite effect upon growth rate. If the alleles of small but opposing effect are gradually eliminated during the course of selection, then the loci of major effect (which have remained heterozygous) will become increasingly more

expressive. As a consequence the genetic variance will appear to increase during inbreeding and selection. A further explanation is that the increased variability resulted from cytoplasmic effects. Maintenance of variability in lines of Schizophyllum commune selected for slow growth rate probably resulted from cytoplasmic variation (Simchen,1966(a);Conolly and Simchem,1968). The possibility that the cytoplasm was responsible,at least in part, for the increased variability during the sexual selection experiment was studied in detail.

One means by which the cytoplasm could be implicated in a cause of variation between oospore cultures would be to compare the oospore progenies of the selection experiment with zoospore progenies derived from the same parents. In instances where the parents were heteroplasmic, oospore and zoospore progenies may be expected to segregate similarly. Innoculum was taken from the one ounce bottles in which the thirteen parents of the selection experiment had been stored, and from each a sample of 24 single zoospore cultures was obtained. The zoospore progenies were termed S2,F2,S3, F3 etc. to correspond with the oospore families derived from the same parents. During the course of the experiment , zoospore progenies S2,S4, and F5 were lost as a result of contamination. The growth rates of the 24 individuals of the remaining 10 progenies were determined in a single experiment (Appendix 3). The

FIG.15 Frequency distribution of zoospore families obtained from the parents selected during the course of the sexual selection experiment. The families are termed F2, S3,F3 etc. to correspond with the oospore families obtained from the same parents.


First generation
F2
S3
F3
F4
S5
S6
F6
S7
F7
1·0
1·5
2·0
2·5
3·0
3·5
GROWTH RATE IN MM PER DAY

growth rate of the parent of each family was also determined in the same experiment. Due to the large size of the experiment only a single growth rate determination was performed on each individual. The growth rate distribution of the 10 zoospore progenies is illustrated in Fig.15. The positions of the arrows in Fig.15 indicate the growth rates obtained for the parent of each family. A discontinuous distribution was detected for two of the ten families. Family S7 segregated 5:18 and family F4 segregated 19:5 for a difference in growth rate. For both families there were no intermediates between the fast and the slow growing single zoospore cultures. Colonies of the fast and slow segregants of family S7 were morphologically similar, but the segregants of family F4 differed. Fast growing zoospore cultures of family F4 produced compact mycelium, while the slow growing zoospore cultures produced diffuse mycelium.

Segregation of the zoospore families S7 and F4 was most probably the result of segregation of cytoplasmic determinants, but it is also possible that the parents of these families had become heterokaryotic during storage and therefore the discontinuous distribution of the growth rates of the families was the result of segregation of the components of a heterokaryon. Second generation zoospore progenies were raised to distinguish these two possibilities.

	ZOOSPORE FAMILY	
PARENT PHENOTYPE	SLOW COLONIES	FAST COLONIES
SLOW	31	0
	30	0
	30	3
FAST	0	33
	0	35
	1	34

Phenotypes of zoospores derived from six individuals of zoospore family S7

	ZOOSPORE FAMILY	
PARENT PHENOTYPE	SLOW COLONIES	FAST COLONIES
SLOW	16	18
	37	9
	33	3
FAST	1	30
	0	33

Phenotypes of zoospores derived from five individuals of zoospore family F4

Table 7.

Zoospore progenies were obtained from six individuals of zoospore family S7 and five individuals of zoospore family F4 and classified according to the fast and slow phenotypes of the family from which they were derived (Table7). Among these 11 second generation zoospore progenies no new phenotypes were observed. Some of the second generation zoospore progenies segregated while others did not. Segregation of the components of a heterokaryon would not be expected to persist through two generations of zoospore propogation. Therefore segregation in zoospore families S7 and F4 was most probably the result of cytoplasmic variation.

Discontinuous cytoplasmic variation of the type observed in zoospore families F4 and S7 could result in both non-heritable and heritable variation between oospores. A cytoplasmicly determined phenotype which segregates on zoospore propogation may also segregate on oospore propogation. Alternatively, an asexually stable cytoplasmically determined phenotype may be stable through oospore propogation and consequently contribute to the response to selection.

It has not yet been established that segregation of cytoplasmic determinants occurred in oospore families F4 and S7. The growth rate distribution of oospore families F4 and S7 was contiuoun and therefore cannot be equated with segregation for growth rate in zoospore families F4 and S7. However, at the time of measurement it was noted that the slower growing

oospore cultures of family F4 produced diffuse mycelium and the faster growing oospore cultures produced dense mycelium although the distinction was not as clear cut as that between zoospore cultures. This suggests that there may have been cytoplasmic entities segregating in oospore family F4. Additional information on this point emerged from the next experiment.

This experiment was designed to further investigate the role of the cytoplasm as a cause of variation between oospore cultures. The phenotypic stability of a sample of single oospore variants through a series of mass hyphal transfers was examined. Persistant phenotypes may be expected to be the result of stable cytoplasmic or stable nuclear determinants whilst those phenotypes which are unstable on mass hyphal transfer are most likely to be cytoplasmicly determined. As a starting point for the mass hyphal transfers, the second oospore family of F4 was chosen (see Fig.14). The two replicate cultures of each of the 20 individuals of this family were taken and arbitrarily labelled R1 and R2. Innoculum was transferred directly from the colonies from which the first set of growth rate measurements had been taken to fresh plates of MM. From the colonies that developed a second set of measurements were taken and so on for two more successive mass hyphal transfers. For the second and

subsequent sets of measurements there were three replicates for each of the 20 single oospore cultures R1, R2 and R3. Innoculum for R1 was derived from R1 of the previous determination and the innoculum for replicates R2 and R3 was derived from R2 of the previous determination. From each set of

SET OF MEASUREMENTS	REPLICATES
FIRST	R1 R2
SECOND	R1 R2 R3
THIRD	R1 R2 R3
FOURTH	R1 R2 R3

measurements (Appendix 4) it was possible to estimate the variation between oospore cultures, the error variation (differences between R2 and R3) and the variation between lines (differences between R1 and $\frac{1}{2}$(R2 + R3)). The two lines were maintained independently throughout the mass hyphal transfers and therefore divergence of the lines reflects spontaneous changes in growth rate. The analysis of variance used to estimate the three components of variation is set out in table 8. Where one or more replicates of an oospore culture were lost through contamination, the remaining replicates for that oospore culture were omitted from the analysis. The

From the three replicates of each individual oospore culture, S, T and U were calculated as :

$$S = R2 + R3 + 2(R1)$$

$$T = R2 + R3 - 2(R1)$$

$$U = R2 - R3$$

For each set of measurements estimated, variances for between oospore cultures ($\hat{\sigma}^2_b$), between lines ($\hat{\sigma}^2_l$) and error ($\hat{\sigma}^2_e$) were calculated as :

ITEM	MS	EMS
Between oospores	$\frac{1}{19}\Sigma(S-\bar{S})^2$	$6\sigma^2_e + 8\sigma^2_l + 16\sigma^2_b$
Between lines	$\frac{1}{20}\Sigma T^2$	$6\sigma^2_e + 8\sigma^2_l$
Error	$\frac{1}{20}\Sigma U^2$	$2\sigma^2_e$

Table 8.

variance components obtained for each consecutive set of measurements were :

SET OF MEASUREMENTS	BETWEEN OOSPORES $\hat{\sigma}^2_b$	BETWEEN LINES $\hat{\sigma}^2_l$	ERROR $\hat{\sigma}^2_e$
FIRST	0.5019	0.0140	0.0140
SECOND	0.3631	0.0265	0.0316
THIRD	0.2653	0.1553	0.0215
FOURTH	0.3151	0.3313	0.0070

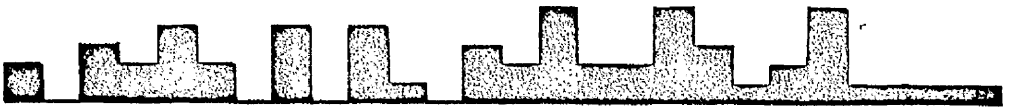
Throughout the series of mass hyphal transfers the differences between R2 and R3 ($\hat{\sigma}^2_e$) remained small and relatively uniform. Variation between oospore cultures was always large but tended to become reduced over successive mass hyphal transfers. The variation between lines was at first similar to the error variation but by the time the fourth set of measurements had been taken the variation between lines was similar to the variation between oospore cultures.

The magnitude of the divergence of the independently maintained lines indicates that considerable spontaneous variation can be released on mass hyphal transfer of single oospore cultures. This however is not evidence that the original oospore phenotypes were largely cytoplasmicly

determined. As the variation between oospore cultures tended to decline with successive transfers the spontaneous changes in growth rate must have on average been towards some common optimal growth rate for the individuals of family F4. Exmmination of the individual growth rates (Appendix 4) revealed that most of the spomtaneous changes resulted in slower growing replicates gaining a faster growth rate and indeed many slow growing colonies were observed to form distinct sectors having a faster growth rate. Presumably, during colony development selection for increased growth rate occurred at the hyphal tip level. If the mass hyphal transfers had been continued to a point at which the variation between oospore cultures ceased to decline then it would have been possible to estimate the extent to which spontaneous variation can alter the growth rates of single oospore cultures of family F4.

It has already been noted that the zoospores derived from the parent of oospore family F4 segregated into two clear cut classes which differed in growth rate and colony morphology. Segregation for the morphological difference also occurred among the cultures of oospore family F4 but the growth rate difference was not detected as the growth rate distribution of family F4 was continuous. During the course of the mass hyphal transfers the form of the growth rate distribution of oospore family F4 altered

Oospore family F4, first measurement.

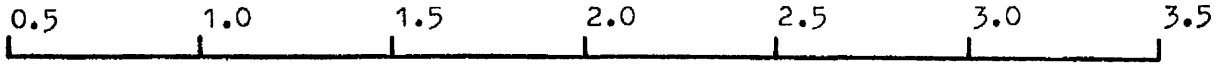


Second measurement.

Third measurement.

Fourth measurement.

Zoospore family F4.



Growth rate (mm/day).

FIG.16, FOUR SUCCESSIVE GROWTH RATE DETERMINATIONS OF OOSPORE FAMILY F4 AND FOR COMPARISON, THE GROWTH RATES OF ZOOSPORES DERIVED FROM THEPARENT OF F4.

(Fig.16). The distribution of the third and fourth set of measurements was clearly bimodal. Furthermore, the two growth rate classes of this distribution correspond closely to the growth rates of the two types of segregant in zoospore family F4. This is taken as strong circumstantial evidence that segregation for a clear cut cytoplasmicly determined growth rate difference occurred on oospore formation in family F4.

Although the oospore cultures of family F4 apparently segregated for a cytoplasmicly determined difference in growth rate the pattern of segregation in young oospore cultures was masked by a background of continuous growth rate variation. This background variation, ~~was however unstable as~~ for the most part, ~~it~~ did not persist through mass hyphal transfer. Here there is an obvious analagy with the behaviour of oospore cultures derived from the fast variant of the asexual selection experiment (Fig.8). Oospores of the fast variant were largely fast but a few non-fast cultures resulted. The non-fast cultures invariably sectored for fast growth rate and after several mass hyphal transfers became typically fast.

Part III Correlated responses to selection in strain P94

A positive response to selection may be

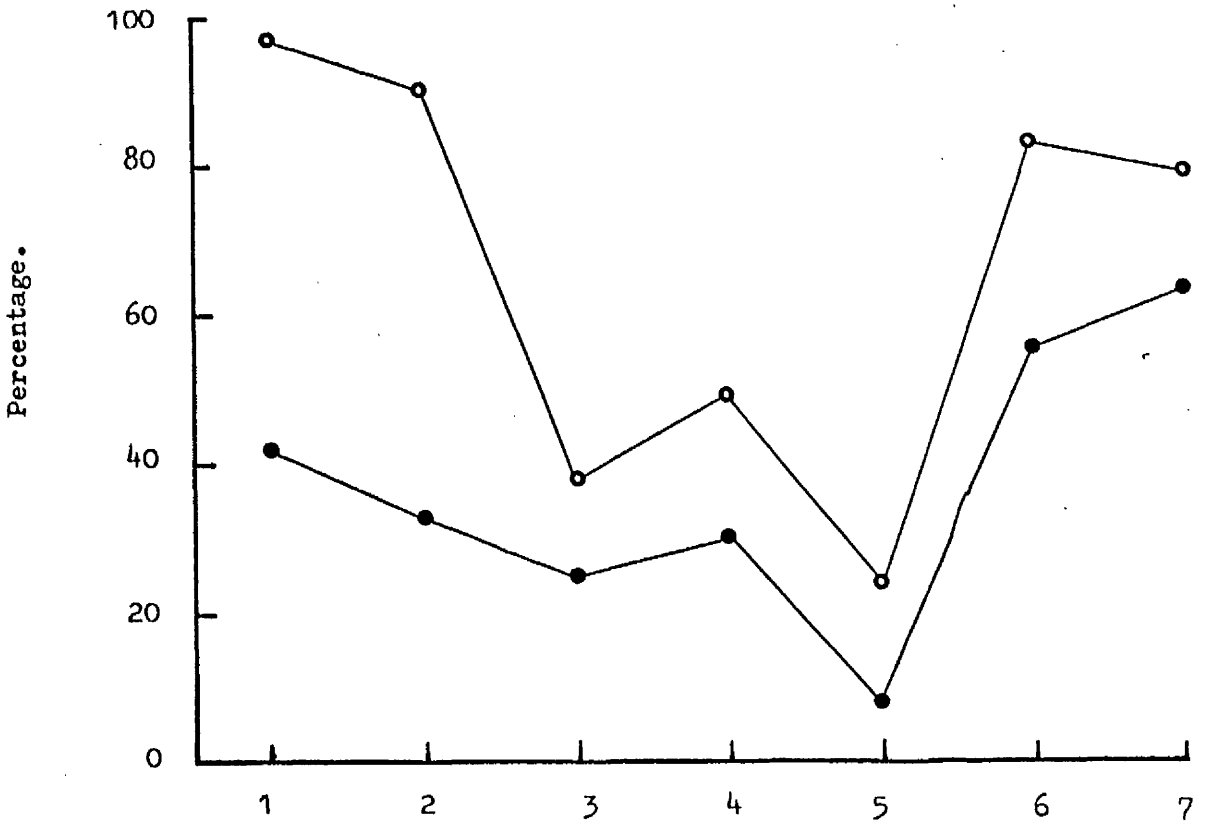
accompanied by a change in the mean expression of characters that are not directly subjected to selection. Such correlated responses may be a pleiotropic expression of the same determinants or may result from linkage associations between the selected determinants and the determinants responsible for the expression of the correlated response. Alternatively, a correlated response may result from natural selection pressures operating on unselected characters during the course of a selection experiment. Selection by means of an inbred line is often accompanied by a deterioration in characters associated with reproductive capacity. Normally such changes as these are and expression of inbreeding depression (Lerner,1954).

Three characters in P.cactorum which are easily assesed for the purpose of detecting a correlated response to selection are percentage germination of oospores, percentage establishment of oospore colonies and fertility.

For each generation of selection it was necessary to isolate a sample of oospores from which to obtain single oospore cultures. Some oospores did not germinate, others germinated and subsequently died and from the remainder single oospore cultures were obtained. From the counts obtained from each sample

FAMILY	NUMBER OF OOSPORES ISOLATED	NUMBER THAT DID NOT GERMINATE	NUMBER THAT DIED AFTER GERMINATION	NUMBER OF COLONIES ESTABLISHED
FIRST GENERATION	77	2	43	32
F2	69	7	39	23
F3	121	75	16	30
F4	63	32	12	19
F5	87	66	14	7
F6	102	17	29	56
F7	91	19	15	57
S2	71	19	23	29
S3	104	7	42	55
S4	74	4	23	47
S5	69	13	21	35
S6	75	7	17	51
S7	47	5	19	23

of oospores the percentage germination and the percentage establishment was calculated and plotted against the generation of selection (Fig.17). For the analysis of variance the percentages were converted to angles. A correlated response for these two characters was not detected in the line selected for slow growth rate. A regression analysis of the percentage germination and the percentage establishment against the generation of selection for slow growth rate was not significant at the 5% level. Percentage germination in the fast selection line declined from



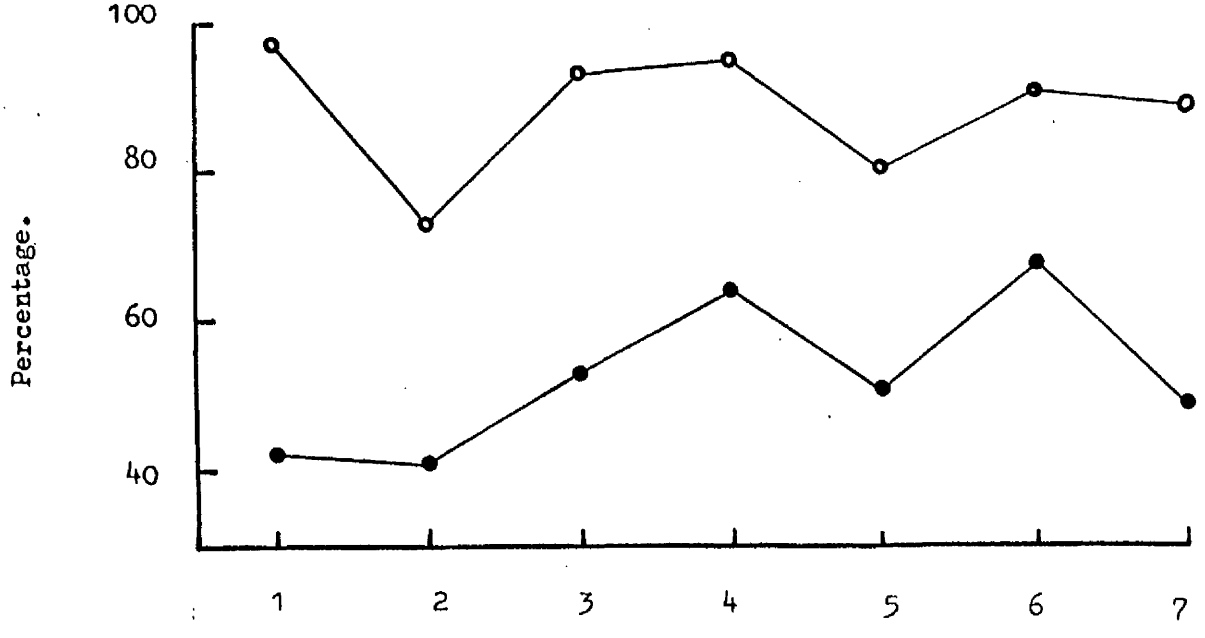


FIG.17, PERCENTAGE GERMINATION (○) AND PERCENTAGE ESTABLISHMENT (●) OF OOSPORES DURING THE COURSE OF SELECTION FOR FAST AND SLOW GROWTH RATE.

the first to the fifth generation (significant at 5%). Percentage establishment in the same line also declined from the first to the fifth generation but the regression MS in this instance was not significant at the 5% level. Both percentage germination and percentage establishment in the fast selection line rose sharply after generation F5 to give relatively high values for generations F6 and F7. Families F6 and F7 were composed of individuals most of which had a cytoplasmicaly determined fast growth rate. It is possible that in families F6 and F7 high percentage germination and percentage establishment are pleiotropic effects of the cytoplasmic determinants which control fast growth rate.

Fertility was determined by oospore counts for the wild type of strain P94 and the parents selected from generations 2,4 and 6 of the fast and slow selection lines. Innoculum of these seven isolates was taken from the one ounce bottles in which the isolates had been stored. For each isolate the oospores from three transects from three Petri dishes were counted (table 9). For plotting (Figs.18 and19) and for the analysis of variance the individual counts were converted to $(x + \frac{1}{2})^{\frac{1}{2}}$. The oospore counts for the fast selection line (Fig. 18) declined with increasing generations of selection for the first three isolates from which counts were obtained, i.e. P94 and the parents selected from F2 and F4. The regression MS

Family from which isolate was derived.							
P94	S2	S4	S6	P94	F2	F4	F6
121	17	9	0	149	43	0	185
207	24	19	0	173	92	0	164
102	21	3	0	140	71	8	185
210	9	14	0	179	123	0	265
257	11	9	0	223	81	0	209
188	13	5	0	123	135	3	252
99	19	13	0	83	96	1	235
102	35	8	0	145	82	5	269
105	13	4	0	139	104	2	307

Table 9. OOspore counts of parents selected during the sexual selection experiment.

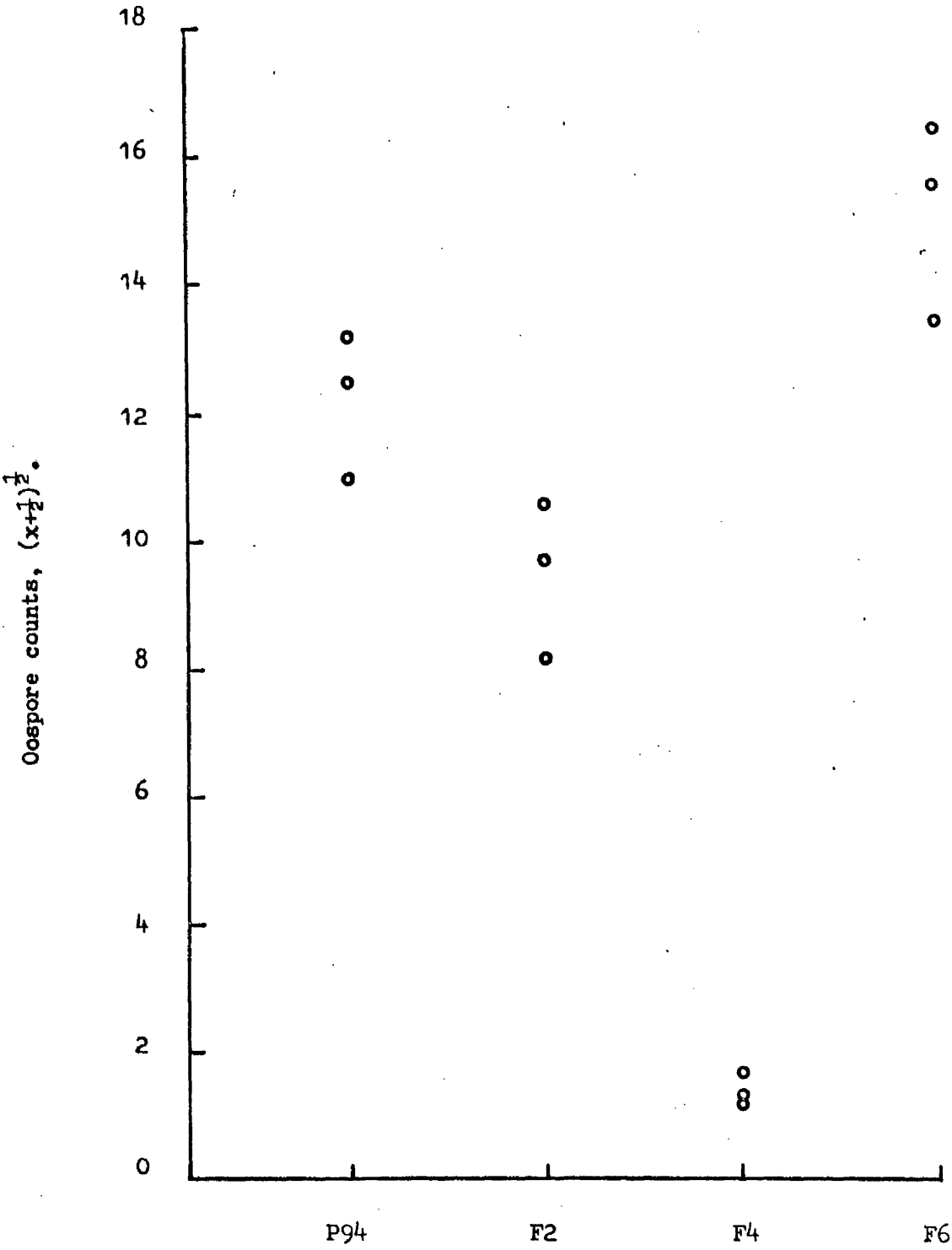


FIG.18, OOSPORE COUNTS OF THE WILD TYPE AND THE PARENTS SELECTED FROM FAMILIES F2, F4 AND F6.

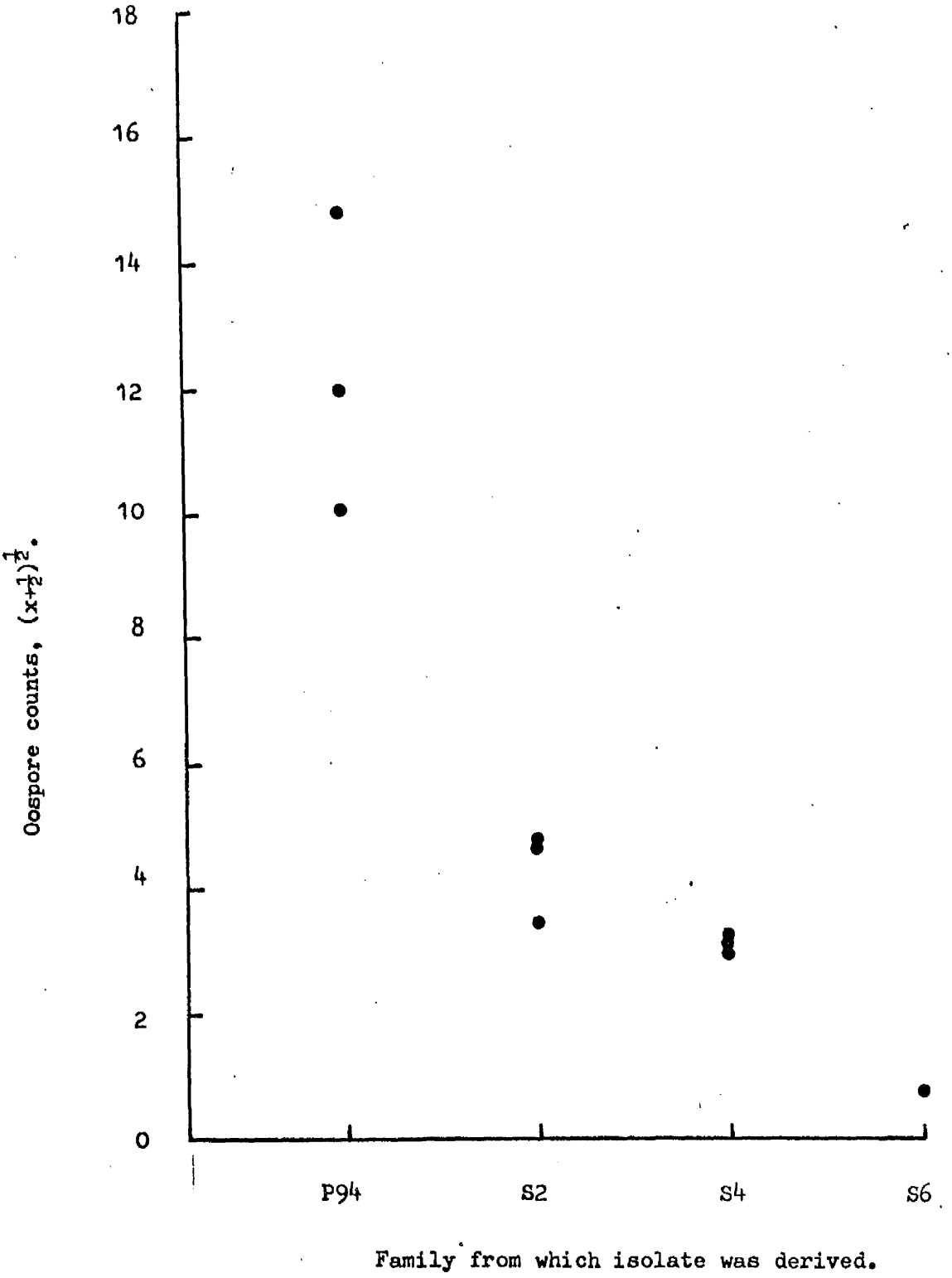


FIG.19, OOSPORE COUNTS OF THE WILD TYPE AND THE PARENTS SELECTED FROM FAMILIES S2, S4 AND S6.

ITEM	SS	df	MS	Variance Ratio
Between isolates				
Regression	522.2750	1	522.2750	191.55 significant at 0.1%
Remainder	43.8571	1	43.8571	
Between Petri dishes	16.3595	6	2.7266	
Between counts	27.5746	18	1.5319	

of the oospore counts for those three parents was significant at the 0.1% level when compared with the between Petri dishes MS. Oospore counts of the parent selected from family F6 were higher than the counts for any other isolate of the fast selection line. They were even higher than the counts obtained from the wild type isolate of P94 (significant at the 10% level). This agrees with the general character of the

ITEM	SS	df	MS	Variance ratio
P94 v F6	38.4214	1	38.4214	6.91 significant at 10%
Between Petri dishes	22.2401	4	5.5600	
Between counts	18.2776	12	1.5231	

parent selected from family F6. It had a cytoplasmicly determined fast growth rate similar to that of the fast variant obtained from the asexual selection experiment. The fast variant was more fertile than

the wild type of P94 (see table 4). It is therefore not surprising that the parent selected from family F6 was also more fertile than the wild type.

The oospore counts of the parents of the slow selection line declined rapidly with progressive generations of selection (Fig.19). The regression MS for the counts was significant at the 0.1% level

ITEM	SS	df	MS	Variance ratio
Between isolates				
Regression	576.6664	1	576.6664	127.4 significant at 0.1%
Remainder	101.7861	2	50.8930	
Between Petri dishes	36.2113	8	4.5264	
Between counts	21.7210	24	0.9050	

when compared with the between Petri dishes MS. The isolate derived from family F6 did not form any oospores on the test plates (MM plus cholesterol) although when grown on SMA plus oat extract sufficient oospores were produced to permit selection to proceed. The marked decline in fertility during selection for slow growth rate was emphasised by the fact that the slowest oospore cultures of families S5 and S6 could not be selected as parents since they did not produce any oospores on SMA plus oat extract.

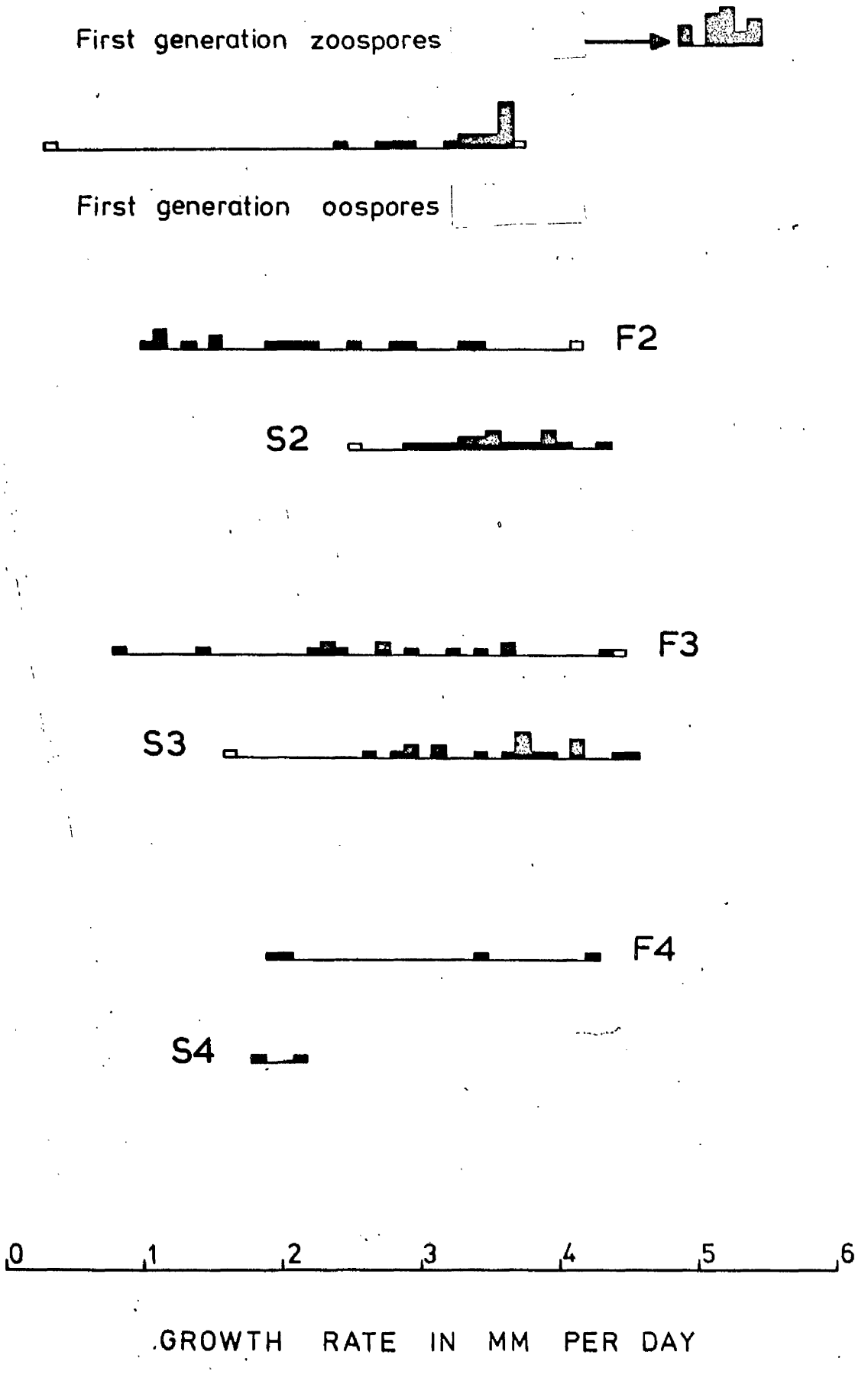
Part IV Results of selection for growth rate in strain P205

The experiments on asexual and sexual variation in strain P205 of P.cactorum were performed to provide results with which to compare the results of the selection experiments on strain P94 of P.cactorum. From the wild type isolate of strain P205 one zoospore family and a number of oospore families were obtained. Selection for fast and slow growth rate from oospore progenies was practiced but the lines had to be discontinued after the fourth generation of selection as at this stage the oospores produced by both lines had an extremely low level of viability.

One first generation zoospore family composed of twenty single zoospore cultures was derived from the wild type of strain P205. For each individual of the family two growth rate determinations were obtained. From the analysis of variance of these growth rates the variation between zoospore cultures was found to be not significant at the 5% level when compared with variation between replicate growth rate determinations (table 10). From the evidence of this single family it would appear that the wild type of strain P205 was neither heterokaryotic nor heteroplasmic for determinants controlling growth rate.

Twenty first generation oospore cultures were also obtained from the wild type of strain P205 and their growth rates determined in duplicate. The

FIG.20 Frequency distribution of a first generation zoospore family and the results of four generations of selection for fast and slow growth rate via oospore propogation of strain P205. The individuals selected as parents for the subsequent generations are unblocked.


First generation zoospores
First generation oospores
F2
S2
F3
S3
F4
S4
0
1
2
3
4
5
6
GROWTH RATE IN MM PER DAY

	ERROR		BETWEEN INDIVIDUALS			
FAMILY	df	MS	df	MS		
First generation zoospores	16	0.0597	19	NS 0.0434	OOSPORES ISOLATED	COLONIES ESTABLISHED
First generation oospores	20	0.0108	19	**** 1.1534	93	73
F2	16	0.0657	16	**** 1.7459	84	20
F3	15	0.0856	14	**** 1.9347	73	15
F4					121	4
S2	18	0.1050	19	*** 0.3344	67	24
S3	20	0.0423	19	**** 1.0018	70	31
S4					82	2

**** significant at 0.1%

*** significant at 1%

NS not significant at 5%

Table 10.

variation between oospore cultures was found to be significant at 0.1% when compared with the variation between replicates (table 10). From a comparison of the mean squares in table 10 and the growth rate distributions in Fig.20 it is clear that oospore progenies of P205 are more variable than zoospore progenies. Selection for fast and slow growth rate was practiced for four generations of oospore propogation (Fig.20). Throughout the course of selection the variation between oospore cultures was significant at 1% and the magnitude of variation remained relatively constant. Although considerable variation between oospore cultures was present a response to selection could not be detected.

The percentage establishment of colonies from isolated oospores declined over the four generations of selection for fast and slow growth rate (table 10). This loss of viability was probably a consequence of inbreeding depression.

Part v Discussion

Selection for fast and slow growth rate by means of oospore propogation of strains P94 and P205 of P.cactorum detected considerable variation between oospore cultures within families. The growth rate variation between oospore cultures was continuous and was greater than the variation between zoospore cultures

of the same strains. In this respect the results agree with those obtained by Boccas (1972) for another homothallic species, P.syringae. Boccas found that oospore cultures of this species were more variable for growth rate than zoospore cultures.

During the course of selection for fast and slow growth rate in strain P94 the variation between oospore cultures gradually increased. The increase in variability was more rapid in the fast line than in the slow line although for the seventh generation of the fast line (F7) a low variance was obtained. It has been argued that the low variance of this family was due to the fixation of a cytoplasmicly determined fast growth rate having the characters of the fast variant encountered during the asexual selection experiment with strain P94. Maintainance of variability or an increase in variability with selection and inbreeding is a common feature of selection experiments. However it is a phenomenon that can not always be readily explained. In the normally outbreeding flowering plant, Lolium perenne, selection for ear emergence accompanied by inbreeding resulted in little, if any, decrease in genetic vaiation over three generations of selection (Cooper,1959). The maintenance of genetic variability was attributed to natural selection operating in favour of heterozygous genotypes. Maintenance of genetic variability has also been observed in inbred lines of Schizophyllum

commune selected for slow growth rate (Simchen,1966(a)). The genetic variance remained high in two lines selected for slow growth rate over 15 and 16 generations respectively without a corresponding response to selection. Evidence was obtained from hyphal tip and duplicate colony experiments to suggest that the variation within the two lines was largely non-nuclear in origin (Connolly and Simchen,1968). Selection for high and low abdominal bristle number in Drosophila melanogaster was accompanied by an increase in variability in both lines (Robertson and Reeve,1952; Clayton,Morris and Robertson,1957). Increased variability in the high line could be explained by the presence of a single locus which, in a heterozygous condition, had a major effect upon increasing bristle number. The effect of this locus was thought to have been enhanced during selection by cross-overs separating from it genes of opposite effect or the presence of 'modifying' genes. During selection for increased and decreased weight in mice variability was maintained at a level equal to or greater than that of the original population (Falconer,1955). Variability in the low selection line rose sharply after the eighth generation. A sudden increase in variability during selection is frequently encountered (Sismanidis,1942; Mather and Harrison,1949;Papa,Srb and Federer,1966) and is often found to result from the breakage of tight linkage relationships between loci heterozygous for

the character under selection.

The increase in variability that resulted from inbreeding and selection in strain P94 was accompanied by a corresponding increase in the magnitude of the response to selection. The response in late generations of selection was greater than the response in early generations of selection. Further, the response was gradual and occurred in both the fast and the slow selection lines. An increase in the magnitude of the response to selection with progressive generations of inbreeding and selection is unusual. More commonly the response declines towards an upper limit beyond which further selection is unsuccessful (e.g. Clayton, Morris and Robertson,1957;Connolly and Simchen,1968; Mather and Harrison,1949;Papa,1971;Pateman,1959; Sismanidis,1942). Presumably, in the present selection experiment with strain P94, the selection limit had not been reached in seven generations of selection.

For the selection experiment with strain P94, the relationship between the response and the accumulated selection differential was linear and gave a mean realised heritability for the fast and slow selection lines of 0.276 $\pm$ 0.050. Thus, a considerable proportion (approximately 72.4%) of the variation between oospore cultures of strain P94 was non-heritable.

Selection for fast and slow growth rate by means of four generations of oospore propogation of strain

P205 was unsuccessful in obtaining a response to selection although considerable variation between oospore cultures was detected. The nature of the non-heritable variation between oospore cultures of P205 was not investigated and selection was not continued beyond a fourth generation as the oospores derived from isolates of the selection lines had, by the fourth generation, an extremely low level of viability.

A correlated response to selection was detected for a number of characters which were not directly subjected to selection. In the fast selection line of strain P94 an increase in growth rate was associated with a decline in the percentage germination of oospores and the percentage establishment of oospore cultures. Oospore counts of representative isolates of the fast and slow selection lines of P94 showed a gradual decline in fertility over increasing generations of selection. As percentage germination, percentage establishment and fertility are characters closely associated with reproductive capacity (fitness characters), the observed changes in their mean values are probably a consequence of inbreeding depression (Lerner, 1954). This view is supported by the fact that the mean value of these characters declined during inbreeding and selection irrespective of whether selection was for fast or for slow growth rate.

Furthermore , selection in strain P205 was accompanied by a reduction in oospore viability even though no response to selection occurred. A deterioration of fitness characters as a consequence of inbreeding depression is a phenomenon of widespread occurrence. It has been demonstrated for many higher organisms e.g. Drosophila subobscura (Hollingsworth and Smith, 1955) and also for some fungi, e.g. Neurospora crassa (Papa, 1971; Pateman, 1959).

The nature of growth rate variation between oospore cultures of strain P94 was studied in detail. Evidence was obtained to suggest that (i) segregation of the components of a heterokaryon, (ii) segregation of nuclear genes and (iii) cytoplasmic variation, was involved in determining the variation between oospore cultures.

Evidence of segregation of the components of a heterokaryon was obtained for the first generation of oospore propogation but this source of variation need not be considered for subsequent generations as the oospores of P.cactorum are uninucleate (Blackwell 1943(a)). Zoospores derived from the wild type of strain P94 exhibited significant variation for growth rate but zoospores derived from single zoospore isolates of the wild type generally did not. Furthermore, second generation zoospore families of the wild type differed in mean growth rate. First

generation oospore cultures derived from the wild type were more variable than the first generation oospore cultures derived from a single zoospore isolate of the wild type. This evidence suggests that the wild type isolate of strain P94 was composed of nuclei differing in their complement of genes that control growth rate . There is no assurance that these unlike nuclei existed in a common cytoplasm as the original isolate of strain P94 was not a single hyphal tip isolate. It is however most likely that the wild type of P94 was heterokaryotic because prior to obtaining first generation oospore and zoospore families the strain was subcultured several times taking innoculum from the colony margin. Significant variation between first generation zoospore cultures of strain P205 was not detected and there is therefore no evidence for heterokaryosis in strain P205.

The evidence that the individual nuclei of strain P94 were heterozygous at loci determining growth rate is rather limited. As it was not possible to distinguish nuclear from non-nuclear variation by menas of a heterokaryon test using suitable nuclear markers the evidence was limited to that which can be obtained from a comparison of oospore and zoospore progenies. First generation oospore cultures of the wild type isolate of strain P94 were more variable than first generation zoospore cultures of the same

isolate. Also first generation oospore cultures of a single zoospore isolate of P94 exhibited significant variation while zoospore cultures of single zoospore isolates of P94 generally did not. The evidence of greater variation between oospore cultures than between zoospore cultures indicates that the nuclei of the wild type isolate of strain P94 were heterozygous at loci controlling growth rate. Boccas (1972) similarly considered heterozygosity to be the source of greater variation between oospore cultures than between zoospore cultures of P.syringae. Recessive mutations affecting growth rate have been induced in P.cactorum (strain IMI 21168) following treatment with N-methyl-N'-nitro-N-nitrosoguanidine and oospores derived from strains carrying the mutations in a heterozygous condition have been observed to segregate in a Mendelian fashion for the growth rate difference (Elliott and MacIntyre,1973).

The evidence implicating the cytoplasm as a factor contributing to the variation between oospore cultures of P94 came from three lines of investigation: (i) an investigation of the effect of storage upon the pattern of segregation among oospore cultures, (ii) the comparison of oospore and zoospore progenies and (iii) an examination of the effect of repeated mass hyphal transfer upon the phenotypic stability of a sample of oospore variants.

The oospore cultures of families F6 and F7 segregated into fast and non-fast cultures. The fast cultures resembled, in growth rate, fertility and colony morphology, the cytoplasmicly determined fast variant encountered during the course of the asexual selection experiment. Evidence that the fast segregants of family F6 and F7 were cytoplasmicly determined came from a comparison of the pattern of segregation in family F6 with a second sample of oospore cultures derived from the same parent, but at a later date. The oospore family obtained on the second occasion had a low variance and was composed of oospore cultures with a fast growth rate. Presumably, the ability of the parent of family F6 to produce segregating progenies was lost during storage. If segregation in the first family of F6 was due to nuclear gene segregation, then all progenies obtained from the parent of this family should segregate similarly. As the second family differed markedly from the first it is probable that the fast growth rate of families S6 andS7 was cytoplasmicly determined.

Zoospores were obtained from each of the parents selected during the course of the sexual selection experiment and compared with the oospore families obtained from the same parents. Unlike the oospore progenies of the selection experiment, most of the zoospore progenies were uniform. However, the zoospores derived from the parents of oospore families

S7 and F4 segregated for a clear cut difference in growth rate. The differences were considered to be cytoplasmicly determined on the evidence of persistant segregation through a further generation of zoospore propogation. The two classes of the segregating zoospore progenies could not initially be correlated with growth rate distributions of oospore families F4 and S7 as these were continuous. However, in the case of oospore family F4, the growth rate distribution became clearly bimodal after three mass hyphal transfers. The two classes of this distribution resembled, in growth rate, the two types of zoospore segregant obtained from the parent of oospore family F4. The similarity in the growth rate distribution of oospore family F4 after three mass hyphal transfers and the growth rate distribution of the zoospores obtained from the same parent provides evidence that the cytoplasmic segregation that occurred at zoospore formation also occurred at oospore formation. If this was the case then it may be inferred that cytoplasmic segregation also occurred on oospore formation in family S7. Furthermore, although undetected, cytoplasmic segregation may also have occurred on oospore formation for other families of the selection experiment. The loss of ability, during storage, of a strain to exhibit segregation for a cytoplasmicly determined difference in growth rate has already been discussed in relation to segregation in family F6.

The twenty single oospore cultures of family F4 were subjected to three successive mass hyphal transfers. Independently maintained lines of mass hyphal transfer diverged indicating that spontaneous changes in growth rate had occured during colony developement. These changes in growth rate were observed on several occasions in individual colonies. Such colonies gave rise to distinct sectors of faster growth rate. The spontaneous changes in growth rate were not random; they tended to result in a closer grouping of the individuals of the family about the two mean values corresponding to the two classes of cytoplasmic segregant of family F4 which have been discussed above. Thus, after three mass hyphal transfers the growth rate distribution of family F4 altered from a continuous distribution to a bimodal distribution. The continuous distribution of young oospore cultures was considered to have resulted from a bimodal distribution superimposed upon a continuous distribution. The continuous growth rate variation of family F4 may have resulted from nuclear segregation, cytoplasmic variation or a combination of these two effects.

The evidence for three sources of variation between oospore cultures has been presented. It appears that,(i) segregation of the components of a heterokaryon,(ii) segregation of nuclear genes and (iii) cytoplasmic variation were involved in determining the variation between the oospore cultures. On the

basis of these three sources of variation it is possible to explain the results obtained from the sexual selection experiment with strain P94. In the first generation of the selection experiment, variation between oospore cultures is visualised as having resulted from nuclear recombination between and within the components of a heterokaryon. In subsequent generations, segregation of nuclear genes probably continued. The increase in variability with selection and inbreeding may have been due to an increase in the importance of cytoplasmic segregation as a cause of variation between oospore cultures. Indeed, the instances of cytoplasmic variation that have been discussed were detected only for later generations of selection. Within each family only two classes of cytoplasmic segregant were observed. From an examination of Figs.9 and 15 it is possible to visualise two classes segregating in the families of generations 4,5,6and 7. The continuous distribution of these families (Fig.9) is thought to have resulted from a masking of a bimodal distribution by contiuous cytoplasmic and/or nuclear variation. A model based on the interelationship of nuclear and cytoplasmic determinants can be constructed to explain the increase in variability with selection and inbreeding. Assume that the cytoplasm of strain P94 contains two types of determinant, i.e. wild type determinants and mutant determinants. A cytoplasm composed predominantly of

wild type determinants will give a wild type phenotype and a cytoplasm composed of predominantly mutant determinants will give a mutant phenotype. To explain the differences between families it must be assumed that the expression of the cytoplasmic determinants alters with the nuclear genotype. Presumably the wild type isolate of P94 contained predominantly wild type determinants and ~~that~~ during asexual growth the ratio of wild type to mutant determinants remained reasonably constant. The occasional formation of a hyphal tip containing predominantly mutant determinants could explain the occurrance of fast growing sectors in wild type colonies. If, during oospore propogation, the mutant determinants multiply at a faster rate than the wild type determinants, then the proportion of mutant to wild type determinants in the cytoplasm will gradually increase during inbreeding and selection. Consequently, as the proportion of mutant determinants increases the proportion of segregants among oospore cultures will also increase. Thus the variation between oospore cultures will increase towards a maximum at which the proportion of mutant to wild type determinants is at an intermediate frequency. Oospore cultures possessing determinants at an intermediate frequency may be phenotypically unstable and selection at the hyphal tip level may result in sectoring and the establishment of a cytoplasm containing predominantly one type of determinant or the other. Presumably the

determinant conferring the faster growth rate will be favoured. This theory could explain the results obtained from the mass hyphal transfers of oospore cultures of family F4. As the proportion of mutant cytoplasmic determinants increases beyond an intermediate frequency the variation between oospore cultures will decrease until no more wild type segregants appear among oospore cultures. This may explain the behaviour of families F6 and F7. Family F6 segregated for a cytoplasmicly determined fast growth rate and the selection of a fast culture resulted in family F7 being composed largely of fast individuals and having a lower variance than F6. Undoubtedly, other explanations can equally account for the nature of the variation between oospore cultures. However, the essential feature of the above argument is that it is theoretically possible to explain the gradual increase in variability that accompanied inbreeding and selection in strain P94 in largely cytoplasmic terms.

The importance of the oospore as a source of variation in wild populations of Phytophthora is virtually unknown (Erwin, Zentmyer, Galindo and Niederhauser, 1963; Gallegly, 1968, 1970(a), 1970(b)). The oospores of strains P94 and P205 of P. cactorum will germinate to nearly 100% when isolated from the parent culture and exposed to light. The oospores of strain P205 will even germinate in situ if the cultures

containing the spores are exposed to light (Elliott and MacIntyre, unpublished results). The magnitude of the variation released on oospore propogation of strains P94 and P205 of P.cactorum has been shown by the present study to be large in comparison with the magnitude of the variation released on zoospore propogation. The readiness with which the oospores of P.cactorum will germinate in culture, the short period of time within which the life-cycle can be completed and the wide range of variation for growth rate that is released on oospore propogation provides evidence that the sexual cycle is important as a mechanism of variation in wild populations of P.cactorum.

Oogonial germination has been observed in P.infestans (Romero and Gallegly, 1963) and in P.palmivora (Boccas, 1970). Also isolated oogonia of strains P94 and P205 of P.cactorum have been observed to germinate (Elliott and MacIntyre, unpublished results). The nuclear state of the products of oogonial germination are unknown. However, if upon germination the processes of meiosis and nuclear fusion are complete, then oogonial germination may provide a means of genetic recombination without the intervention of oospore formation. It would be interesting to discover the significance of oogonial germination.

APPENDIX I

Duplicate growth rate measurements (mm/day) on twenty zoospores of each family of the asexual selection lines.

	First generation F1		F2		F3	
1.	1.72	1.63	1.74	1.76	2.86	2.86
2.	1.81	1.76	1.88	1.80	2.86	2.89
3.	1.75	1.72	1.80	1.82	2.86	2.73
4.	1.76	1.65	1.76	1.86	2.98	2.89
5.	1.87 *	1.92	1.80	1.82	2.70	2.86
6.	1.84	1.84	1.74	1.72	2.73	2.86
7.	1.76	1.78	1.72	1.82	2.92	2.79
8.	1.76	1.69	1.74	1.74	2.83	2.95
9.	1.82	1.76	1.72	1.84	2.86	2.92
10.	1.72	1.71	1.78	1.86	2.83	2.79
11.	1.79	1.89	1.68	1.76	2.86	2.92
12.	1.78	1.66	1.72	1.84	2.92 *	3.01
13.	1.72	1.73	1.82	1.76	2.92	2.79
14.	1.62	1.66	1.86	1.68	2.86	2.83
15.	1.66	1.69	1.78	1.72	2.89	2.76
16.	1.62 *	1.60	1.78	1.74	2.70	2.92
17.	1.72	1.73	1.82	1.72	2.95	2.98
18.	1.66	1.66	1.82	1.66	2.89	2.79
19.	1.68	1.69	1.66 *	2.16	2.89	2.83
20.	1.68	1.79	1.78	1.68	2.73	2.76

* selected individuals

contd...

APPENDIX I contd.

	F4		F5		F6	
1.	2.45	2.57	2.56	2.44	2.68	2.65
2.	2.70	2.77	2.53	2.58	2.57	2.48
3.	2.67	2.60	2.58	2.56	2.43	2.60
4.	2.57	2.47	2.44	2.58	2.71	2.65
5.	2.57	2.52	2.50	2.58	2.63	2.51
6.	2.82	2.62	2.58	2.67	2.65	c
7.	2.65	2.72	2.73	2.64	2.68	2.63
8.	2.57	2.67	2.67	2.61	2.65	2.74
9.	2.52	2.65	2.58	2.56	2.57	2.60
10.	2.65	2.67	2.56	2.53	2.63	2.63
11.	2.85 *	2.70	2.76	2.50	2.68	2.68
12.	2.65	2.75	2.53	2.64	2.68	2.71
13.	2.62	2.72	2.70 *	2.67	2.57	2.65
14.	2.50	2.50	2.61	2.61	c	2.71
15.	2.38	2.35	2.50	2.50	2.68	2.65
16.	2.60	2.42	2.53	2.58	2.63	2.54
17.	2.72	2.75	2.56	2.44	2.71 *	2.71
18.	2.42	2.70	2.67	2.47	2.63	2.68
19.	2.67	2.57	2.58	2.61	2.65	2.71
20.	2.35	2.38	2.58	2.61	2.71	2.68

c replicate lost through contamination

* selected individuals

contd...

APPENDIX I contd.

	F7		F8		S2	
1.	2.50	2.66	2.99	3.13	1.84	1.84
2.	2.78	c	3.04	3.18	c	1.84
3.	2.97 *	2.94	3.04	3.01	1.80	1.80
4.	2.75	2.62	3.04	3.13	1.86	1.82
5.	2.84	2.94	c	3.18	1.82	1.80
6.	2.72	2.59	3.10	3.07	1.80	1.82
7.	2.78	2.75	c	3.10	1.86	1.80
8.	2.87	2.75	3.21	3.01	1.80	1.92
9.	2.62	2.84	2.96	3.10	1.84	1.82
10.	2.56	2.91	3.10	3.07	1.86	1.78
11.	2.87	2.59	2.90	3.04	1.84	1.80
12.	2.81	2.53	3.04	3.07	1.86	1.80
13.	2.87	2.81	2.87	2.96	c	1.96
14.	2.72	2.47	2.99	3.10	1.78 *	1.76
15.	2.72	2.75	c	3.13	1.78	1.72
16.	2.84	c	3.07	2.96	1.86	1.86
17.	2.75	2.59	c	3.10	c	1.76
18.	2.62	2.97	3.13	3.07	1.84	1.88
19.	2.91	2.91	3.07	3.01	1.82	1.80
20.	c	2.66	3.07	3.13	1.84	1.80

c replicate lost through contamination

* selected individuals

contd...

APPENDIX I contd.

	S3		S4		S5	
1.	1.74	1.69	1.57	1.60	1.48	1.51
2.	1.74	1.74	1.55	c	1.54	1.54
3.	1.82	1.74	1.60	1.57	1.48	1.68
4.	1.79	1.77	1.55	1.55	1.48	1.49
5.	1.74	1.77	1.53	c	1.46	c
6.	1.85	1.72	1.51	1.57	1.46	1.51
7.	1.74	1.74	1.58	1.53	1.51	1.53
8.	1.74	1.72	1.57	1.53	1.48	1.49
9.	1.69 *	1.72	1.53	1.55	1.48	1.46
10.	1.72	1.72	1.55	1.53	1.44	1.48
11.	1.97	1.77	1.84	1.57	1.48	1.48
12.	1.74	1.69	1.60	1.53	c	1.48
13.	1.74	1.74	1.55	1.53	1.51	1.49
14.	1.74	1.77	1.51	1.57	1.46	1.46
15.	1.79	1.72	1.51 *	1.53	1.53	c
16.	1.79	1.69	c	1.58	1.49	c
17.	1.72	1.72	1.91	1.76	1.46	1.48
18.	1.74	1.69	1.68	1.51	1.46	1.46
19.	1.77	1.72	1.53	1.53	1.44 *	1.43
20.	1.69	1.79	1.53	1.51	1.49	c

c replicate lost through contamination

* selected individuals

contd...

APPENDIX I contd.

	S6		S7		S8	
1.	1.64	1.62	1.77	c	1.95	1.98
2.	1.62	1.62	1.69	1.69	1.95	2.00
3.	1.62	1.64	1.64	1.66	2.09	c
4.	2.64	2.59	1.66	1.64	c	1.92
5.	1.67	1.57	1.77	1.67	2.00	c
6.	2.49	2.52	1.67	1.62	2.12	2.00
7.	2.82	2.54	1.66	1.64	2.00	2.09
8.	1.64	1.67	c	*1.60	2.09	1.95
9.	c	2.96	1.66	1.71	c	2.06
10.	2.49	2.49	1.66	1.67	1.95	2.00
11.	1.75	1.77	1.67	1.71	2.12	1.98
12.	1.69	1.77	1.64	1.67	2.12	1.98
13.	1.72	1.75	1.60	1.67	2.00	1.92
14.	1.62	1.62	c	1.75	1.92	1.86
15.	1.67	c	1.69	1.60	2.00	1.95
16.	1.69	1.69	1.62	1.64	1.98	2.09
17.	3.47	2.72	1.69	1.69	2.37	1.98
18.	1.59	*1.62	c	1.60	2.06	2.03
19.	1.64	1.67	1.66	1.67	2.03	c
20.	1.67	1.62	1.69	1.66	1.95	1.95

c replicate lost through contamination

* selected individuals

APPENDIX II

Duplicate growth rate measurements (mm/day) on twenty oospores of each family of the sexual selection lines.

	First generation F1		F2		F3	
1.	2.30	2.02	1.69	1.69	1.71	1.64
2.	2.30	2.25	1.62	1.69	1.55	1.58
3.	2.16	2.27	1.46	1.57	c	1.67
4.	2.06	2.09	1.53	1.64	1.58	1.55
5.	2.11	1.95	1.55	1.57	c	1.58
6.	2.41	2.27	1.68	1.62	1.71	1.67
7.	1.97 *	2.02	1.60	1.57	1.62	1.58
8.	1.92	2.16	1.68	1.71	1.62	c
9.	1.92	1.87	1.60	1.55	1.62	1.62
10.	2.04	2.04	1.62	1.59	1.62	1.76
11.	1.97	1.99	1.57	1.61	1.67	1.62
12.	1.87	1.95	1.71 *	1.79	1.60	1.58
13.	2.06	2.09	1.73	1.71	c	1.85
14.	1.90	1.90	1.51	1.57	1.71	1.69
15.	2.06	1.87	1.59	1.60	c	* 2.03
16.	2.09	2.09	1.59	1.57	1.62	1.67
17.	1.85	1.85	1.50	1.46	1.87	1.98
18.	2.13 *	1.97	1.64	c	1.80	1.74
19.	1.90	c	1.55	1.59	1.67	1.64
20.	1.95	1.99	1.55	1.62	c	1.62

c replicate lost through contamination

* selected individuals

contd...

APPENDIX II contd.

	F4		F5		F6	
1.	1.62	1.58	2.02	1.99	1.44	1.44
2.	1.19	1.25	1.41	1.47	2.91	2.80
3.	1.02	0.98	2.05	2.14	0.74	0.74
4.	1.07	1.39	2.88 *	2.72	2.06	1.99
5.	1.95	2.01	2.14	2.02	2.10	2.03
6.	1.82	1.82	2.39	2.92	1.07	0.66
7.	2.01	1.95	1.29	2.20	2.88	2.91
8.	1.07	1.21	2.30	2.88	2.76	2.80
9.	1.21	0.98	2.17	1.68	1.92	1.33
10.	2.48 *	2.42	1.99	1.99	2.88	2.76
11.	1.09	1.00	2.54	2.60	2.29	2.14
12.	2.30	2.07	2.14	1.90	1.73	1.59
13.	1.07	1.00	1.41	1.44	2.99 *	2.95
14.	1.23	1.19	1.50	1.81	2.76	2.84
15.	1.31	1.52	2.08	2.11	2.69	2.88
16.	0.98	0.92	2.02	1.87	0.92	0.92
17.	1.72	1.82	2.11	2.20	2.76	2.80
18.	1.62	1.87	1.65	1.59	2.91	2.80
19.	1.29	1.13	2.08	2.02	c	2.91
20.	c	c	1.38	c	1.25	1.14

contd...

APPENDIX II contd.

	F7		S2		S3	
1.	3.10	3.07	1.56	1.51	1.46	1.50
2.	3.07	3.07	1.36 *	1.45	1.66	1.68
3.	3.03	2.93	1.52	1.51	1.52	1.56
4.	2.93	3.00	2.37	2.12	1.54	1.52
5.	3.07	3.16	1.54	1.61	1.54	1.58
6.	3.07	3.10	1.52	1.52	1.09 *	1.09
7.	2.97	2.97	1.52	1.54	1.54	1.52
8.	2.69	2.90	1.56	1.56	1.52	1.52
9.	2.97	2.97	1.56	1.58	1.54	1.54
10.	3.03	3.00	1.54	1.56	1.52	1.50
11.	1.75	1.91	1.58	1.52	1.52	1.54
12.	3.16	2.93	1.60	1.51	1.54	1.52
13.	3.10	2.97	1.49	1.52	1.52	1.54
14.	3.07	2.93	1.67	1.63	c	1.52
15.	2.97	3.16	1.54	1.52	1.62	1.62
16.	3.13	3.03	1.56	1.83	1.54	1.52
17.	3.23	3.03	1.58	1.54	1.54	1.54
18.	3.00	3.03	1.52	1.51	1.70	1.70
19.	2.97	2.87	1.47	1.56	c	1.68
20.	3.10	3.00	c	c	1.62	1.60

contd...

APPENDIX II contd.

	S4		S5		S6		S7	
1.	1.12	1.12	1.52	1.52	1.76	1.76	1.65	1.68
2.	1.14	1.51	1.45	1.48	1.97	1.94	0.92	0.89
3.	1.67	c	1.09 *	1.05	1.65	1.65	1.98	1.91
4.	1.12	1.14	c	1.50	1.73	1.79	1.75	1.88
5.	c	1.18	1.45	1.50	1.42	1.45	0.82	0.68
6.	1.20	1.16	1.59	1.61	1.65	1.65	1.35	1.42
7.	1.48	1.50	2.02	1.98	1.62	1.62	1.19	1.09
8.	1.16	1.14	1.59	1.61	1.76	1.82	1.98	2.08
9.	1.08	1.08	1.50	1.50	1.71	1.62	1.71	1.78
10.	1.32	1.28	1.43	1.48	1.76	1.76	1.22	1.25
11.	1.18	1.22	1.43	1.39	1.47	1.47	1.58	1.68
12.	1.06 *	1.08	1.57	1.64	0.58	0.95	1.55	1.45
13.	1.16	1.14	1.41	1.39	1.16 *	1.24	1.48	1.42
14.	1.20	1.14	1.14	1.14	1.27	1.30	0.73	1.35
15.	1.48	1.44	1.25	1.30	1.42	1.45	1.02	1.05
16.	1.65	1.59	0.91	0.89	1.76	1.79	1.75	1.75
17.	1.71	1.63	1.59	1.70	1.42	1.42	0.96	0.96
18.	1.57	1.57	1.50	1.52	1.21	1.24	1.88	1.81
19.	1.16	1.16	1.64	1.64	1.42	1.39	1.98	1.98
20.	1.16	1.14	1.61	1.55	1.01	1.04	c	1.55

APPENDIX III

Growth rates (mm/day) of zoospore progenies of some isolates obtained from the sexual selection experiment.

Fast selections

Family from which isolate was selected	FIRST GENERATION	F2	F3	F5	F6
Growth rates of isolate	2.67	3.00	2.96	3.22	3.19
	2.59	2.56	2.81	3.19	3.22
Growth rates of single zoospore cultures of each isolate	2.56	2.81	1.70	3.22	3.19
	2.30	2.93	2.81	3.07	3.11
	2.26	2.85	1.56	3.33	3.07
	2.04	2.93	1.81	3.15	3.15
	2.48	2.78	1.85	3.26	3.26
	2.44	2.59	1.96	3.22	3.33
	2.41	2.67	2.93	3.15	3.15
	2.52	2.67	1.89	3.37	3.07
	2.44	2.74	1.30	3.44	3.19
	2.48	2.67	1.56	2.93	c
	2.52	2.63	1.48	3.30	3.19
	2.63	2.70	1.26	3.11	3.19
	2.70	c	1.74	3.07	3.44
	2.52	2.52	1.78	3.15	3.44
	2.52	2.81	1.85	3.04	3.44
	2.44	2.67	1.96	3.22	3.41
	2.59	2.85	1.56	3.48	3.26
	2.52	2.63	1.44	3.30	3.19
	2.41	2.81	2.78	3.26	3.48
	2.63	c	1.56	3.19	3.41
	2.56	2.93	1.67	3.44	3.30
	2.59	2.63	1.85	3.22	3.26
	2.37	2.63	2.74	3.19	3.33
	2.52	2.74	2.78	c	3.07

contd....

APPENDIX III contd

Slow selections				Wild type
S2	S4	S5	S6	P94
2.04	1.22	1.81	1.44	2.48
2.00	1.15	1.89	1.37	2.63
1.89	1.19	1.81	1.15	2.52
1.89	1.22	1.89	1.96	2.37
1.89	1.11	1.89	1.19	2.26
2.04	1.19	1.89	1.89	2.15
1.85	1.22	1.96	1.78	2.63
2.04	1.11	1.93	1.78	c
2.00	1.15	1.81	1.81	2.33
2.07	c	2.04	1.78	2.44
1.93	1.15	1.89	1.85	2.19
1.89	1.22	1.93	1.78	c
1.93	1.22	1.81	2.00	2.41
1.96	1.26	1.89	1.81	2.48
1.96	1.15	1.89	1.89	2.15
1.81	1.30	c	1.26	2.52
1.81	1.30	1.96	1.93	2.22
1.96	1.19	1.85	1.96	2.41
2.00	1.30	1.89	1.89	2.30
1.96	1.30	1.89	1.19	2.48
2.00	1.26	1.96	1.85	2.52
1.93	1.26	1.85	1.81	2.48
1.96	1.19	1.93	1.85	2.48
2.00	1.19	1.93	1.22	2.48
1.93	1.22	2.11	1.81	2.52
1.93	1.22	1.85	c	c

APPENDIX IV

Four consecutive growth rate determinations (mm/day) on twenty oospore colonies of family F4 of the sexual selection experiment.

See page for an explanation of replicates 1, 2, and 3.

	First		Second		
	1	2	1	2	3
1.	2.21	2.14	2.78	2.28	2.42
2.	1.54	1.36	1.45	1.45	1.70
3.	1.07	0.96	1.99	1.27	1.05
4.	1.79	2.07	1.99	e	1.30
5.	1.43	1.61	1.48	1.70	1.08
6.	1.14	1.18	1.99	1.55	1.52
7.	2.39	2.46	2.53	2.71	2.64
8.	1.36	1.50	1.48	1.27	1.23
9.	1.93	1.82	2.35	2.46	2.28
10.	3.04	3.25	3.51	3.14	3.22
11.	2.50	2.46	2.49	2.53	2.46
12.	1.46	1.71	2.28	1.95	2.06
13.	2.61	2.36	3.14	3.00	2.75
14.	1.07	1.29	2.31	2.82	2.42
15.	2.82	2.57	3.36	e	2.67
16.	3.18	3.00	3.43	e	3.11
17.	1.11	1.21	1.77	1.70	1.34
18.	1.64	e	2.93	3.00	2.82
19.	2.61	2.61	2.75	2.75	2.53
20.	3.14	3.11	e	3.11	3.11

contd...

APPENDIX IV contd.

	Third			Fourth		
	1	2	3	1	2	3
1.	3.39	2.54	c	3.11	c	3.18
2.	1.71	1.50	1.39	2.82	1.00	1.18
3.	3.11	0.89	1.04	3.00	0.54	0.54
4.	1.86	2.25	1.96	2.11	1.64	1.46
5.	1.61	1.21	1.64	1.29	1.36	1.43
6.	1.82	1.86	1.96	3.43	1.93	1.75
7.	2.50	2.50	2.61	2.54	2.54	2.57
8.	1.50	1.29	1.32	1.04	0.93	1.14
9.	2.25	2.75	2.71	2.61	2.61	2.71
10.	3.21	3.39	3.57	3.21	3.07	3.18
11.	2.54	2.39	2.61	2.43	2.43	2.39
12.	2.11	2.07	2.04	1.54	1.82	1.68
13.	2.79	2.93	2.71	2.75	3.04	2.93
14.	2.79	2.71	c	2.82	2.71	2.57
15.	3.00	2.96	c	3.00	2.96	2.89
16.	3.19	3.43	c	3.11	3.18	3.21
17.	1.57	1.82	1.39	1.21	0.93	1.07
18.	2.86	c	c	2.68	c	2.82
19.	2.50	2.54	2.50	2.29	2.43	2.39
20.	3.04	3.11	3.18	3.04	2.96	3.00

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